



Hadyoon, Charlotte Sara (2003) *Controlled and tailored modification of polymer interfaces for use in biosensing systems*. PhD thesis.

<http://theses.gla.ac.uk/2615/>

Copyright and moral rights for this thesis are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the Author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the Author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

# **Controlled and Tailored Modification of Polymer Interfaces for use in Biosensing Systems**

by

**Charlotte Sara Hadyoon**

Thesis submitted for the degree of

**Doctor of Philosophy**

to the

Department of Electronics and Electrical Engineering

Faculty of Engineering

**University of Glasgow**

May, 2003

© C. S. Hadyoon, 2003



## *Abstract*

Biosensors based on conducting polymers appear particularly well suited to the requirements of modern biological analysis. Electrodeposited conducting polymers have found widespread use in the development of sensing membranes. For biosensing applications it is often desirable to immobilise biomolecules securely on the electrode surface.

The research described here was performed to develop and characterise modified conducting polymers suitable for use in the development of biosensor arrays. The research pursued centred around a post polymer-deposition modification strategy based on nucleophilic substitution of the pentafluorophenol group of the polymerised pyrrole derivative, pentafluorophenyl 3-(pyrrol-1-yl) propanoate (PFP). The activated ester present within this derivative is as an ideal reaction site for amine terminated species.

Initially, electrochemical polymerisation growth conditions were determined and controlled to produce homopolymer and copolymer films with different structural and electrochemical characteristics. These polymer films were subsequently modified through various chemical reactions (e.g. with biotinylated species) to produce templates that could be used in biosensor developments. Furthermore, an important aspect in the development of a biosensing interface is the minimisation of non-specific adsorption and to that end a strategy was developed that involved modifying poly(PFP) films with poly(propyleneglycol) motifs. Usefully, an XPS based technique was developed to determine the extent of adsorption of labelled biological macromolecules on the modified poly(PFP) surfaces.

Significantly, towards the development of a multianalyte biosensing substrate, a method was developed to control the reaction of solution based amine terminated species with the homopolymer poly(PFP). This involved electrochemically doping the polymer film to inhibit/ promote nucleophilic reaction with amine containing species. Preliminary examples are given of the application of this technique was to micropattern species on multi-digitated electrodes.

## *Acknowledgements*

Firstly I would like to thank Prof. Jon Cooper for giving me the opportunity to perform this research within the Bioelectronics department. Without the help, advice and support given throughout the years, completion of this research would certainly not have been possible.

Thanks too to Dr. Dave Morris for his guidance during my degree and for giving me the opportunity to face the challenge of undertaking a PhD. I would like to take this opportunity to wish him good luck on his retirement.

I would like to express my sincere gratitude to Dr. Andrew Glidle. Your guidance, knowledge and advice have been invaluable and it is greatly appreciated. Thank you for putting up with me over the years!

I would also like to say thanks to the friends I have made whilst working in the Bioelectronics Department: Craig Young, Stephen Hamill, Todd Kleckner, Mary Robertson, Scott McKendry, Craig Walker, Tim Drysdale, Harold Chong and Duncan Ross.

I would like to thank those friends who have supported me whilst doing my PhD (I know you have been subjected to a considerable amount of moaning): Victoria Campbell, Lynn Grainger, Shirley Walker and Nigel Crook.

Special thanks goes to Ross Campbell- you have seen all the highs and lows of doing a PhD and without your encouragement this would have never been completed.

Lastly my biggest thanks goes to my family:

To my wee sissy, Farah, you have been a big support over the years and I will never forget how much you have helped me (I have no excuse now not to tidy the bedroom!).

To my brothers, David and Cameron, for the endless “entertainment”!

Finally, to my mum and dad for their kindness and support. I appreciate all you have done for me and without you this would have never been possible.

In answer to the question I have heard so often- “That’s it finished!”

## ***Publications***

1. Hadyoon, C. S., Glidle, A., Morris, D. G., Cooper, J. M., “ Electrochemically controlled micropatterning of immobilised species on functionalised electrode interfaces”, *Chemical Communications*, vol. 17, pp. 1683, 1999.
2. Glidle, A., Hadyoon, C. S., Cass, A. E. G., Cooper, J. M., “ Modifications of electrodeposited polymers by ion chelation to produce templates for biomolecule immobilisation”, *Electrochimica Acta*, vol. 45, no. 22, pp. 3823, 2000.
3. Glidle, A., Bailey, L., Hadyoon, C. S., Hillman, A. R., Jackson, A., Ryder, K. S., Saville, P. M., Swann, M. J., Webster, J. R. P., Wilson, R. W., Cooper, J. M., “Temporal and spatial profiling of the modification of an electroactive polymeric interface using neutron reflectivity”, *Analytical Chemistry*, vol. 73, no. 22, pp. 5596, 2001.
4. Glidle, A., Swann, M. J., Hadyoon, C. S., Cui, L., Davis, J., Ryder, K. S., Cooper, J. M., “XPS assaying of electrodeposited copolymer composition to optimise sensor materials”, *Journal of Electron Spectroscopy and Related Phenomena*, vol. 121, no. 1-3, pp. 131, 2001.



# Contents

<b>Abstract</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Publications</b>	<b>iv</b>
<b>Contents</b>	<b>v</b>
<b>Abbreviations</b>	<b>x</b>
<b><i>Chapter 1 Preparation of Conducting Polymers for Biosensor Technology.....</i></b>	<b><i>1</i></b>
<b>1.1 Introduction .....</b>	<b>1</b>
1.1.1 Biosensors .....	1
1.1.2 Conducting Polymers for Preparation of Biosensors .....	4
1.1.2.1 Preparation of Conducting Polymers.....	4
1.1.3 Functionalisation of Conducting Polymers .....	7
1.1.3.1 Chemical Modification of a Preformed Polymer.....	7
1.1.3.2 Polymerisation of Monomer Derivatised with the Biomolecules .....	7
1.1.3.3 Polymerisation of Monomers with Activated Leaving Groups .....	9
1.1.4 Patterning of Electrodes with Polymeric Adducts .....	11
<b>1.2 Strategy of Bio-Polymeric Functionalisation.....</b>	<b>12</b>
<b>1.3 Outline of Thesis Structure.....</b>	<b>13</b>
<b>1.4 References .....</b>	<b>14</b>
<b><i>Chapter 2 Electrochemical Methods used for the Development and Characterisation of a Polymer System.....</i></b>	<b><i>21</i></b>
<b>2.0 Introduction .....</b>	<b>21</b>
<b>2.1 Materials .....</b>	<b>22</b>
<b>2.2 Electrochemical Methods .....</b>	<b>25</b>
2.2.1 Cyclic Voltammetry.....	26

2.2.1.1 Background.....	26
2.2.1.2 Instrumentation .....	29
2.2.1.3 Potential Control of Post Deposition Functionalisation.....	31
2.2.2 Potential Step Chronoamperometry .....	33
2.2.2.1 Background.....	33
2.2.2.2 Polymerisation of Polymer Films.....	34
2.2.3 Electrochemical Quartz Crystal Microgravimetry .....	37
2.2.3.1 Background.....	37
2.2.3.2 Instrumentation .....	38
2.2.3.3 The Use of EQCM Gravimetry for Subsequent Immobilisation Reactions .....	39
<b>2.3 Illustrative Results from the Application of the Electrochemical Techniques.....</b>	<b>41</b>
2.3.1 Cyclic Voltammetry to Polymerise Poly(PFP).....	41
2.3.2 Use of CV to the Polymerise Poly(Py/PFP) Copolymer Films.....	45
2.3.3 EQCM Measurements During the Polymerisation of Poly(PFP).....	47
<b>2.4 Summary.....</b>	<b>56</b>
<b>2.5 References .....</b>	<b>57</b>

## ***Chapter 3 Spectroscopic Methods used for the Development and Characterisation of a Polymer System..... 58***

<b>3.0 Introduction.....</b>	<b>58</b>
<b>3.1 Materials .....</b>	<b>59</b>
<b>3.2 Spectroscopic Methods .....</b>	<b>59</b>
3.2.1 X-ray Photoelectron Spectroscopy (XPS) .....	59
3.2.1.1 Background.....	59
3.2.1.2 XPS Imaging <sup>7-10</sup> .....	65
3.2.2 Reflectance-Adsorption Infra Red Spectroscopy (RAIRS) .....	65
3.2.2.1 Background.....	65
<b>3.3 Results and Discussion.....</b>	<b>67</b>
3.3.1 XPS Analysis of PFP Homopolymers.....	67
3.3.1.1 Comparison of Homopolymers Prepared Using Different Electrochemical Techniques.....	70
3.3.2 XPS Analysis of PFP/Py Copolymers.....	70
3.3.2.1 Methods Used to Establish Copolymer Compositions.....	71

3.3.2.2 Comparison of Copolymers Prepared Using Different Electrochemical Techniques ..	73
3.3.3 Use of XPS for the Analysis of Immobilisation Reactions.....	74
3.3.3.1 XPS to Analyse Immobilisation of Copolymer Interfaces .....	76
3.3.4 Application of RAIRS Spectroscopy to Homopolymer Analysis .....	78
3.3.5 Application of RAIR Spectroscopy to Copolymer Analysis .....	80
3.3.6 Application of RAIR Spectroscopy for Analysis of Polymer Reactions .....	81
3.4 Summary.....	84
3.5 References.....	85
 <i>Chapter 4 Detailed Characterisation of the Reaction Between Poly(PFP) Electrodes and Simple Amines.....</i>	 <i>88</i>
4.0 Introduction .....	88
4.1 Materials .....	89
4.2 Results and Discussion.....	91
4.2.1 Reaction of Polymer with Ferrocene Ethylamine .....	91
4.2.1.1 Reaction of Poly(PFP) Modified Electrodes with Ferrocene Ethylamine.....	92
4.2.1.2 General Model for the Reaction of Polymer Film with Solution Species .....	94
4.2.1.3 Reaction of PFP/Py Copolymer Films with Ferrocene Ethylamine.....	106
4.2.2 Reaction with Hexylamine .....	110
4.2.2.1 Poly(PFP) Homopolymer Reaction with Hexylamine.....	111
4.3 Summary.....	115
4.4 References.....	116
 <i>Chapter 5 Development of a Method to Control the Modification of Poly(PFP) Electrodes.....</i>	 <i>117</i>
5.0 Introduction .....	117
5.1 Materials .....	119
5.2 Results and Discussion.....	119
5.2.1 Control of Reaction of Ferrocene Ethylamine with Poly(PFP).....	119
5.2.2 Control of Reaction of Hexylamine with Poly(PFP).....	123
5.2.3 Selective Patterning of Ferrocene Ethylamine on Poly(PFP) Modified Electrodes .....	124

5.2.4 Patterning of Biological and Electron Transfer Motifs on Poly(PFP) .....	127
5.3 Summary .....	130
5.4 References .....	130
<b>Chapter 6 Reaction of Cystamine Modified Poly(PFP) Polymers.....</b>	<b>131</b>
6.0 Introduction .....	131
6.1 Materials .....	133
6.2 Results and Discussion.....	133
6.2.1 Production of Cystamine Modified Polymer .....	133
6.2.1.1 Characterising the Bonding in Homopolymer Reaction with Cystamine .....	133
6.2.1.2 Copolymer Reaction with Aqueous Cystamine.....	138
6.2.2 Production of Cystamine-Platinum Template.....	142
6.2.2.1 Cystamine Modified Polymer Reaction with Platinum .....	143
6.2.2.2 Subsequent Immobilisation Forming Platinum Complex .....	146
6.2.3 Potential Control of Poly(PFP) Reaction with Cystamine.....	152
6.2.4 Control of Formation of Cystamine and Platinum Complex .....	155
6.3 Summary.....	156
6.4 References .....	158
<b>Chapter 7 Interaction of Biotin Modified Poly(PFP) Films with Streptavidin and Streptavidin-Enzyme Conjugates .....</b>	<b>160</b>
7.0 Introduction .....	160
7.1 Materials .....	161
7.2 Results and Discussion.....	162
7.2.1 Reaction of Biotin Modified Poly(PFP) Film with Streptavidin.....	162
7.2.1.1 Reaction of Homopolymer with Pentylamine Biotin.....	163
7.2.1.2 Reaction of Biotin Modified Polymer with Streptavidin .....	164
7.2.1.3 Reaction of Biotin Modified Polymer with Streptavidin Conjugates .....	167
7.2.2 Reaction of Cystamine Modified Poly(PFP) with Biotin Derivative Followed by Immobilisation of Streptavidin and Streptavidin Conjugates.....	169



7.2.2.1 Reaction of Cystamine Modified Poly(PFP) Film with I-PEO-acetylbiotin .....	171
7.2.2.2 Reaction of Modified Film with Streptavidin Conjugates .....	172
7.2.3 Reaction Control of Poly(PFP) with Cystamine Prior to Biotin-Streptavidin Modification	174
7.2.4 Selective Patterning of Biotin-Streptavidin Modified Poly(PFP) .....	176
7.3 Summary.....	181
7.4 References.....	181
 <i>Chapter 8 Non-specific Binding of Modified Poly(PFP) Films .....</i>	 <i>183</i>
8.0 Introduction.....	183
8.1 Materials .....	184
8.2 Results and Discussion.....	184
8.2.1 Immobilisation of PPG on Poly(PFP) Film.....	184
8.2.2 Non-specific Binding Studies.....	186
8.3 Summary.....	192
8.4 References .....	193
 <i>Chapter 9 Conclusion and Further Work .....</i>	 <i>195</i>
9.1 Conclusion.....	195
9.2 Further Work.....	198

## ***Abbreviations***

<b>AP</b>	Alkaline Phosphatase
<b>CV</b>	Cyclic Voltammetry
<b>DCC</b>	Dicyclohexylcarbodiimide
<b>DMSO</b>	Dimethyl Sulphoxide
<b>DHU</b>	Dicyclohexylurea
<b>EQCM</b>	Electrochemical Quartz Crystal Microgravimetry
<b>FeCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub></b>	Ferrocene Ethylamine
<b>HRP</b>	Hydrogen Peroxidase
<b>IPA</b>	Isopropyl alcohol
<b>MeCN</b>	Acetonitrile
<b>MP-11</b>	Microperoxidase-11
<b>PEG</b>	Poly ethylene glycol
<b>PFP</b>	Pentafluorophenyl 3-(pyrrol-1-yl) propanoate
<b>PPG</b>	Bis-(2-aminopropyl) propylene glycol
<b>Poly(PFP)</b>	Homopolymer of Pentafluorophenyl 3-(pyrrol-1-yl) propanoate
<b>Poly(Py/PFP)</b>	Copolymer of Pentafluorophenyl 3-(pyrrol-1-yl) propanoate and Pyrrole
<b>Poly(Py)</b>	Homopolymer of Pyrrole
<b>Py</b>	Pyrrole
<b>PyCOOH</b>	(pyrrol-1-yl) propanoic acid
<b>QCM</b>	Quartz Crystal Microbalance
<b>RAIRS</b>	Reflectance-Adsorption Infra Red Spectroscopy
<b>St</b>	Streptavidin
<b>St-AP</b>	Streptavidin-Alkaline Phosphatase Conjugate
<b>St-HRP</b>	Streptavidin-Hydrogen Peroxidase Conjugate
<b>TEAP</b>	Tetraethylammonium perchlorate
<b>TOA</b>	Take-off angle (degrees)
<b>XPS</b>	X-ray Photoelectron Spectroscopy

# Chapter 1

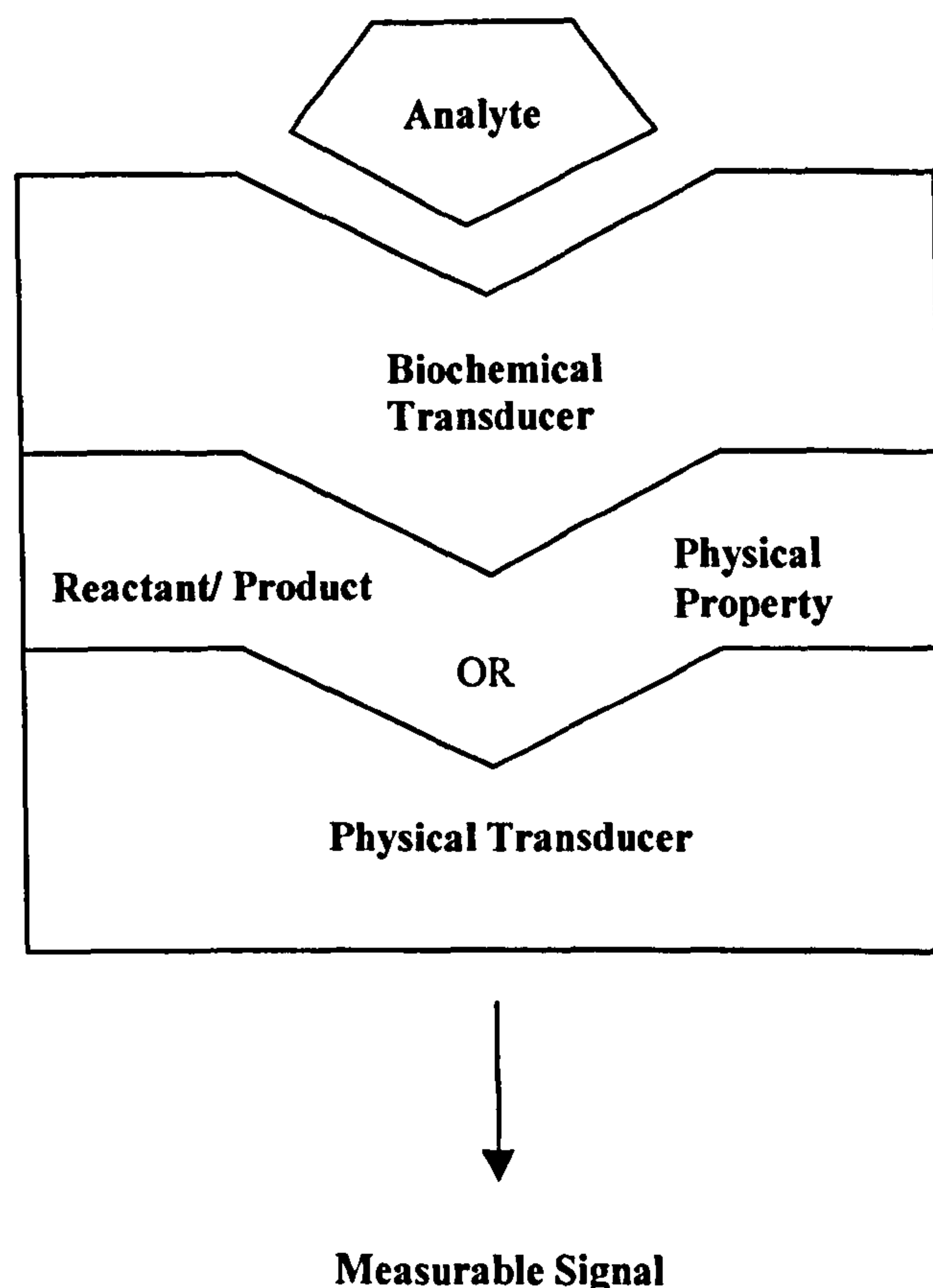
## *Preparation of Conducting Polymers for Biosensor Technology*

### *1.1 Introduction*

#### **1.1.1 Biosensors**

A biosensor is an analytical device that incorporates/ detects biological species, which are either immobilised or retained in functional contact with a transducer surface in order to monitor the local environment.<sup>1</sup> The fundamental characteristic of such a device is that the biological material should be able to produce a quantitative signal in response to an interaction with a specific analyte. There are a wide variety of biological materials, transducer types and sensing mechanisms that can be incorporated into a biosensor. For example, enzymes, antibodies and nucleic acids are all suitable as biologically-sensitive materials, capable of producing a specific recognition reaction for a particular analyte.<sup>2-10</sup> The types of sensing strategy and transducer that are presently being developed to convert the biological reaction into a measurable response include electrochemical (potentiometry, amperometry, conductimetry and voltammetry)<sup>11</sup>, optical (fluorescence, chemiluminescence and bioluminescence)<sup>12</sup>, piezo-electric, acoustic and immunological techniques.<sup>1, 6, 13-21</sup> However, irrespective of the sensing method, the biologically functionalised transducer should be able to fulfil the following criteria: high specificity for the analyte of interest, fast response times, quantitative response, stable over the concentration and temperature ranges required and, in some instances, be adaptable to a miniaturised format.

Biosensors based on conducting polymers appear particularly well suited to the requirements of modern biological analysis.<sup>22</sup> Such polymers are attractive materials with which to spatially define sensitive layers on the surface of an electrode (or indeed other sensors). This is due to the ease of electrodeposition on the electrode surface and possibility of functionalisation or entrapment with biological components.



**Figure 1.1:** Schematic Diagram of a typical biosensor.

One strategy used in the construction of electrochemical biosensor is based on the entrapment of biomolecules in polymer films during the electropolymerisation process on the electrode surface.<sup>23-25</sup> This method involves the application of an appropriate potential to the working electrode whilst immersed in an aqueous solution of the appropriate monomer, electrolyte, solvent and enzyme. Enzymes present in the immediate vicinity of the electrode surface are thus incorporated in the growing polymer.



The advantage of the electrochemical polymerisation is that films can be prepared easily in a rapid one-step procedure. Furthermore, this method enables exact control of the thickness (ranging from nm to many microns) of the polymer layer based on the measurement of the electrical charge passed during the electrochemical polymerisation.

Most of the electrochemically deposited polymer films used for the biomolecule immobilisation are conducting polymers for example, polythiophene, polyaniline, polyindole and polypyrrole. Owing to their conductivity, the thickness of the polymer film can be easily varied (and is not restricted, as is the case for very thin films of non-conducting organic polymers). Nevertheless, a number of biosensors based on insulating electropolymerised films like polyphenols, poly (ortho-phenylenediamine) and over oxidised polypyrrole have been investigated. In those systems, the growth of the polymer films is self-limiting and therefore the resulting biosensor may exhibit fast response time. Nevertheless, the conducting polymers, primarily polypyrrole and its derivatives, play a predominant role due to their versatile applicability and the wide variety of molecular species covalently attached to pyrrole.

An alternative to simple biomolecule entrapment within a electrodeposited film is the binding of a biomolecule to polymer films bearing reactive groups,<sup>26-28</sup> a strategy which involves electropolymerisation followed by covalent attachment. The advantage of using this procedure lies in the possibility to use optimal conditions for each step. In particular, the initial formation of polymer films can be performed under ideal conditions (e.g. suitable organic solvents and high potential values for polymerisation) which are generally incompatible with biomolecules. Furthermore, the covalent binding of the biomolecule to the polymer can be carried out in aqueous solutions containing additives and stabilisers, which preserve the catalytic activity and/ or the recognition properties of the biomolecule.

In addition to the direct functionalisation of conducting polymers by covalent binding of biomolecules to polymer films, the attachment of biomolecules by both adsorption affinity binding have also been utilised in biosensor research. An example

is the attachment of biomolecules to sensor surfaces via the formation of avidin-biotin bridge using biotinylated biomolecules or avidin or streptavidin- conjugated biomolecules. Due to the high affinity of the interaction, this coupling method has been extensively used for biomolecule immobilisation.<sup>29</sup>

Furthermore, research has been carried out into the modification of electrodeposited polymers by incorporating suitable metal ions in the electrode matrix where immobilisation of biomolecules involves both ionic and ligand binding interactions. For example, a method has been developed to immobilise proteins within conducting polymers through the specific interaction of polymer-localised metal ions, an example of which is the Ni chelation to a polymer resin that has the ability to bind his-tagged modified proteins (proteins with 6 histadine residues).<sup>30</sup>

### **1.1.2 Conducting Polymers for Preparation of Biosensors**

Since the early development of enzyme electrodes a great deal of attention has been paid to developing chemically modified electrodes. Those of interest in this study are electrodes, which are coated with conducting polymers for preparation of biosensors. In general these polymers possess the conjugated  $\pi$  electron systems, and, characteristically, they are both easily reduced and oxidised reversibly, and can be grown to different thickness'.

Due to these characteristics, the conducting polymer component of a polymer-based biosensor can be used for two purposes. Firstly, since they are electronically conductive in certain situations, they can, via an appropriate electron transfer mechanism, transduce chemical reactions to electrical signals. The second use, which is utilised during this research, is that they can be prepared with functionally to provide “soft” matrices for the biomolecule immobilisation.

#### **1.1.2.1 Preparation of Conducting Polymers**

The conducting polymers used for chemical and biological sensors are generally formed from films of an electroconductive polymer grown on metal or

semiconductor electrodes. Usually, oxidative polymerisation is the method used to produce polymers used in biosensor preparations <sup>31-34</sup> (see Figure 1.2 for an example). Unlike simple physisorbed monolayers, which are not particularly stable and can only hold a limited amount of surface bound species, polymer growth can be used to fabricate films on an electrode surface of arbitrary thickness. This method of preparation is easy to perform, is controllable and leads to the formation of insoluble cross-linked conducting polymers in very intimate contact with the electrode. Usefully, conventional spectroscopic and microscopy tools can be employed to characterise the polymer developed in polymer biosensor research. For example infrared spectroscopy, UV-Vis, X-ray photoelectron spectroscopy and the various scanned probe microscopies are widely used.

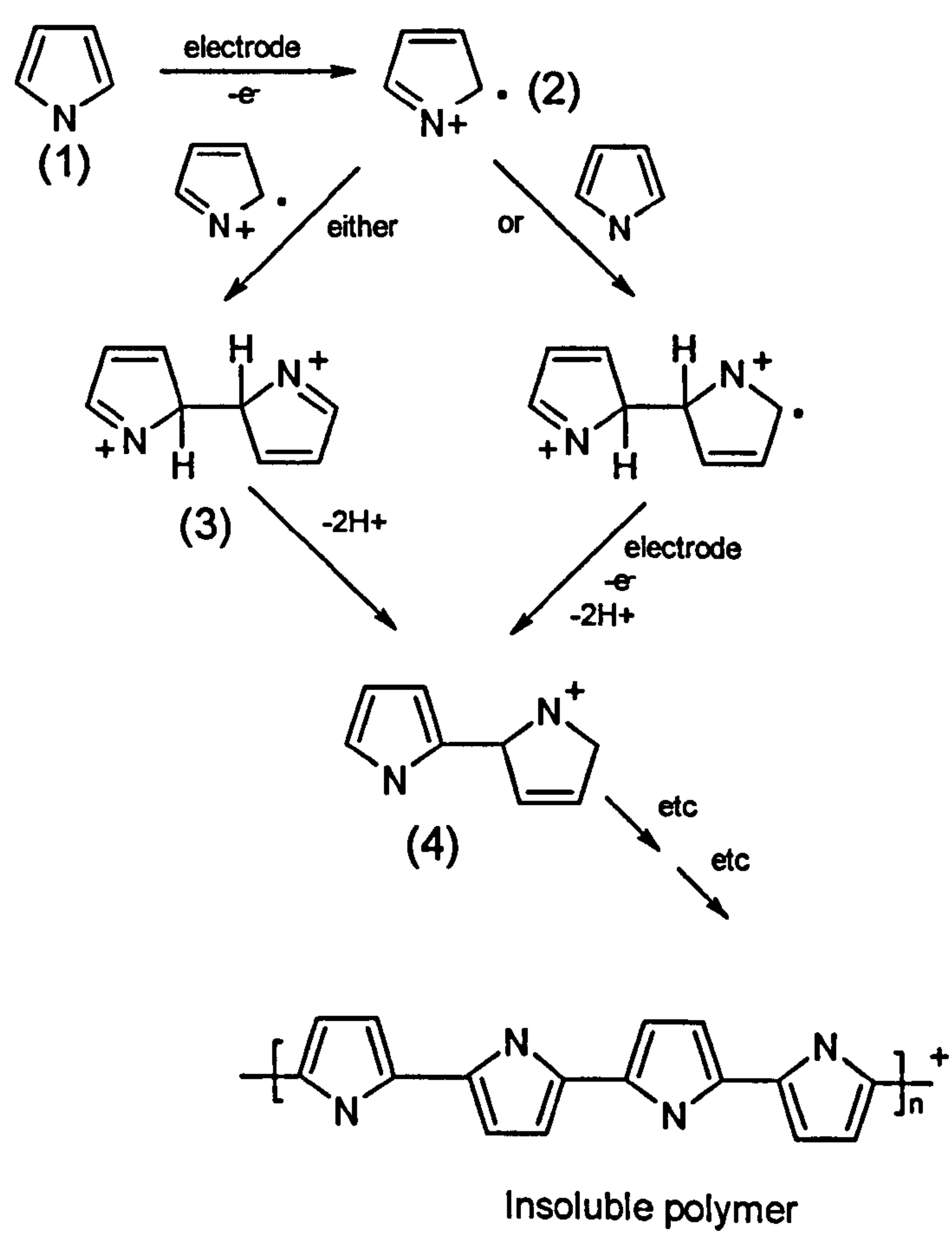
The morphology and composition of homopolymers and copolymers produced by electrochemical polymerisation can be varied using potentiostatic (potential step chronoamperometry), galvanostatic or potentiodynamic methods (cyclic voltammetry). When using the galvanostatic method of formation, an amount of current is passed through the solution containing the monomer and the polymer film is formed on a working electrode. In the potentiostatic method the working electrode is kept at a potential at which polymerisation takes place on the electrode. In addition, cyclic voltammetry is often used, in which the potential is scanned across a range of voltages determined by consideration of the oxidation potential of the relevant monomer species. In this latter case alteration of the rate at which the potential is scanned has a critical dependence on the flux, and the rate at which the monomers reach the electrode.

Potentiostatic and cyclic voltammetry formation of polymer films have a wider application in biosensor fabrication than the galvanostatic methods, and, therefore, both of these methods are used to develop the pyrrole-based homopolymers and copolymers used in this research (discussed in greater detail in Chapter 2). <sup>11</sup>

Poly(pyrrole) has been known for over 50 years and is one of the most studied electroactive polymers and was first synthesised by electrochemical polymerisation in 1968. The mechanism of its synthesis (Figure 1.2) has also been studied



extensively.<sup>35</sup> The first step of the mechanism involves the oxidation of the pyrrole monomer (1) to give a radical cation (2). This radical cation can either react with a second radical cation to give the dimeric species (3) or react with the neutral monomer molecule, followed by further oxidation of the dimer to give (3). Further oxidation and coupling reactions of this type lead to oligomers and eventually to the deposition of the polymer at the electrode since the solubility of the oligomers decreased with increasing chain length and entanglement. Due to its hydrophilic properties (compatible with the outer shells of proteins, for example), it has often been used in constructing biosensors.<sup>1</sup> In early studies a variety of functionalised poly(pyrrole) films were also used for biosensor preparation, providing a method of tailoring the local polymeric environment, and hence controlling protein-polymer interaction. The methods used to prepare these functionalised polymers are discussed in Section 1.1.3.



**Figure 1.2:** Mechanism of electrochemical polymerisation of pyrrole.



### **1.1.3 Functionalisation of Conducting Polymers**

#### **1.1.3.1 Chemical Modification of a Preformed Polymer**

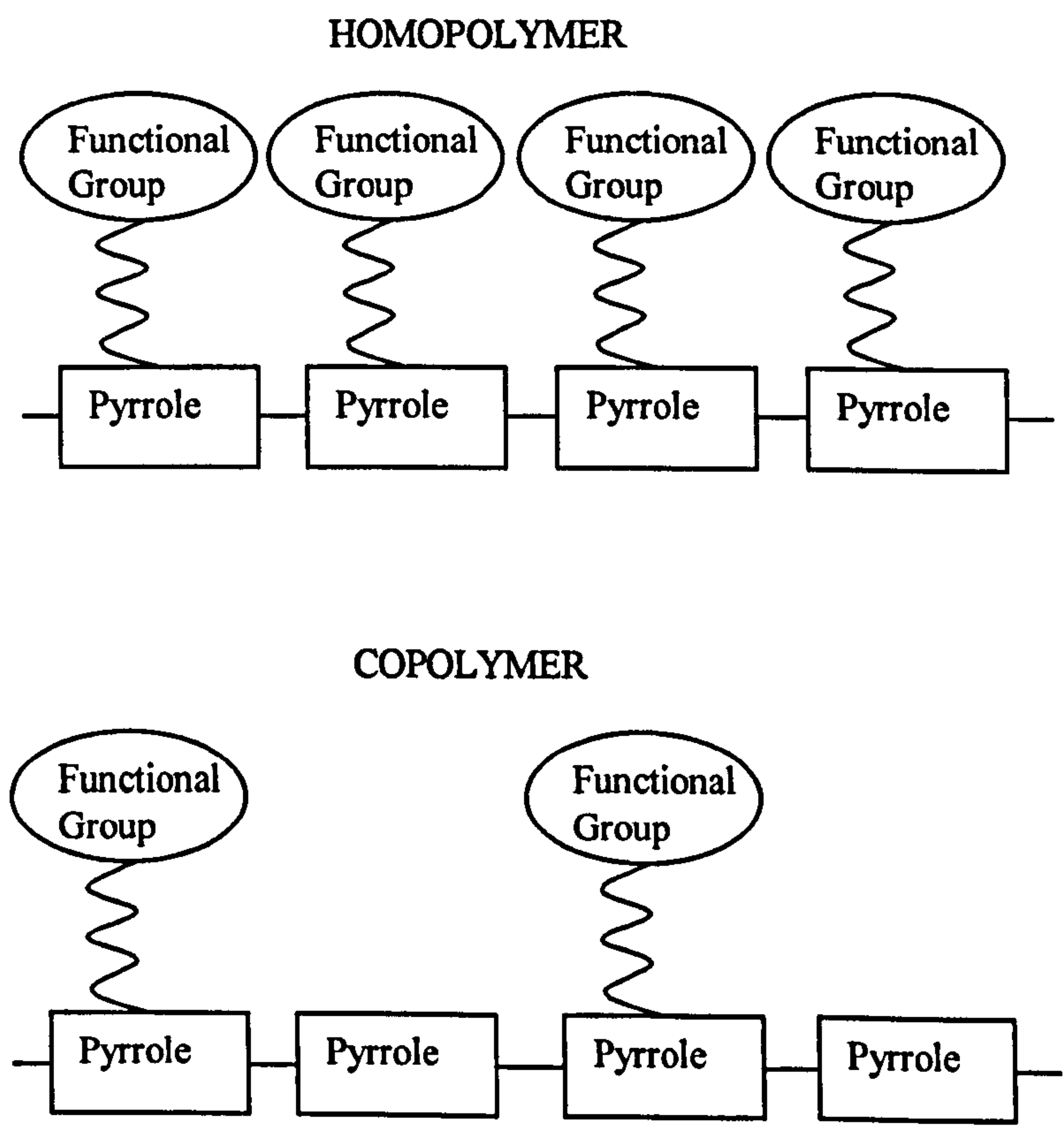
Chemical modifications of a formed functionalised polymer backbone are possible using covalent linking methods to specifically attach the desired molecules. Depending on the system, this attachment site is either at the polymer membrane surface or the polymer chains in the bulk. These chemical reactions performed on the polymer lead to the introduction of reactive groups within the polymer, which can then be reacted with appropriate biomolecules, such as enzymes, to produce the desired polymer surface. Examples have been reported which use chemically modified polymer films with a facile leaving group to achieve covalent attachment of redox enzymes and DNA.<sup>36-39</sup>

#### **1.1.3.2 Polymerisation of Monomer Derivatised with the Biomolecules**

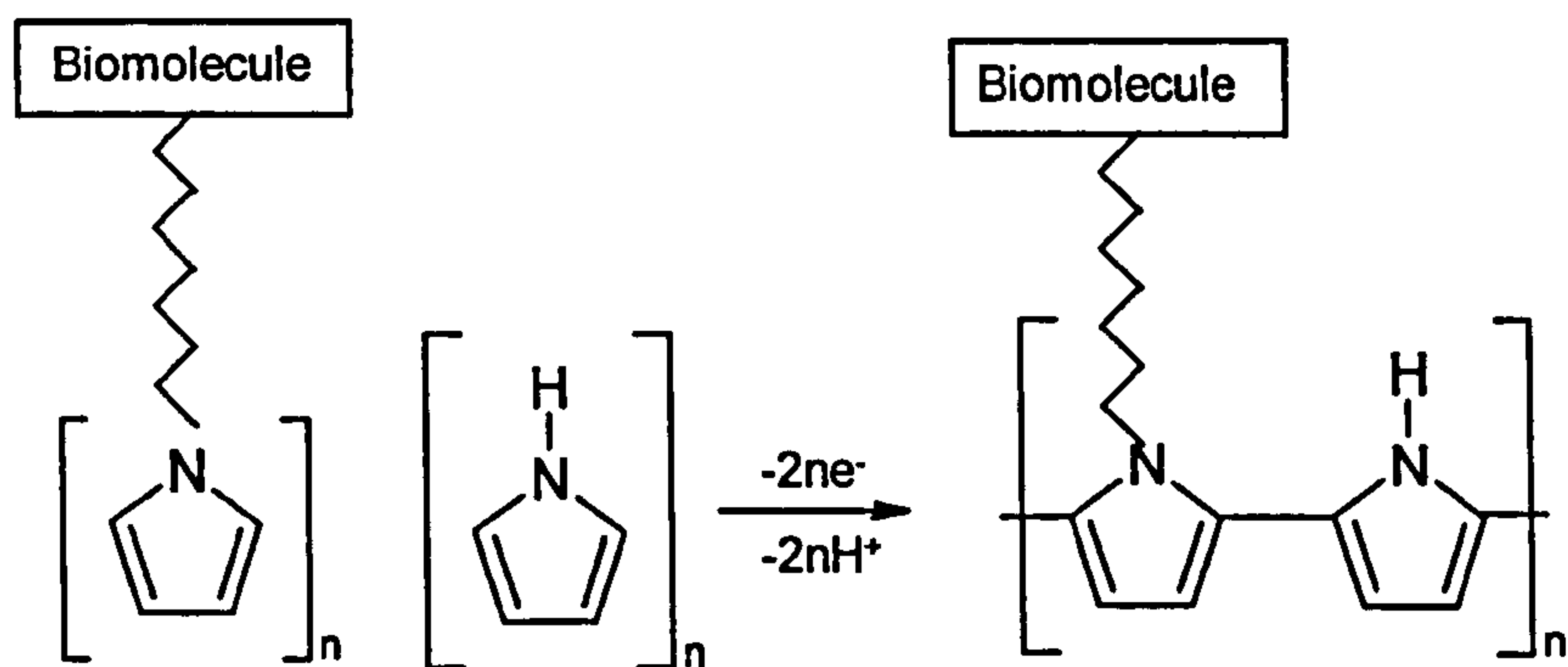
A further method of biopolymer preparation is the polymerisation of biomolecule modified electroconductive monomers. Whilst using this strategy, it is important to assess whether polymerisation is likely to be prohibited or compromised by the bulky structure of the modified monomer species.<sup>44</sup> Notably, utilising this technique, pyrrole films containing covalently immobilised glucose oxidase have been produced.<sup>40, 41</sup> During the synthesis of the monomer species, specific chemistry was carried out to conjugate a pyrrole derivative with the primary amine sites of the lysine residues of the enzyme. It was not possible to produce homopolymer films using this single monomeric species, due to the steric interactions, during polymerisation of the bulky, functionalised monomer units. However, it was possible to copolymerise the monomer-modified enzyme with free pyrrole (non-functionalised) to produce a copolymer film (see Figures 1.3 and 1.4). Consequently, this reduced interactions between adjacent functional groups of the polymer chain. In addition, the covalently immobilised enzyme film showed both higher enzyme activities and larger amperometric response to glucose compared to physically entrapped enzyme films.<sup>42</sup>

Similarly biotinylated pyrrole derivatives have been formed by reaction of 3-(pyrrol-1-yl) propanoic acid to the free primary amine of pentylamine or 5-(biotinamido) pentylamine.<sup>43</sup> Copolymerisation of pyrrole with the biotin derivative produced a copolymer with available biotin sites. These sites then served as the point of attachment of streptavidin or avidin in the subsequent performance of biotin/streptavidin/ biotin type.

Various problems can be encountered using this technique, which include difficulties encountered during the chemical synthesis of the functionalised monomers (i.e. purification) and the use of costly chemicals. Furthermore, it is important for the biomolecules to retain their activity after polymerisation has occurred. It is also possible that the polymerisation process may render the attached biomolecules inactive and, therefore, unsuitable for the use in biosensor technology.



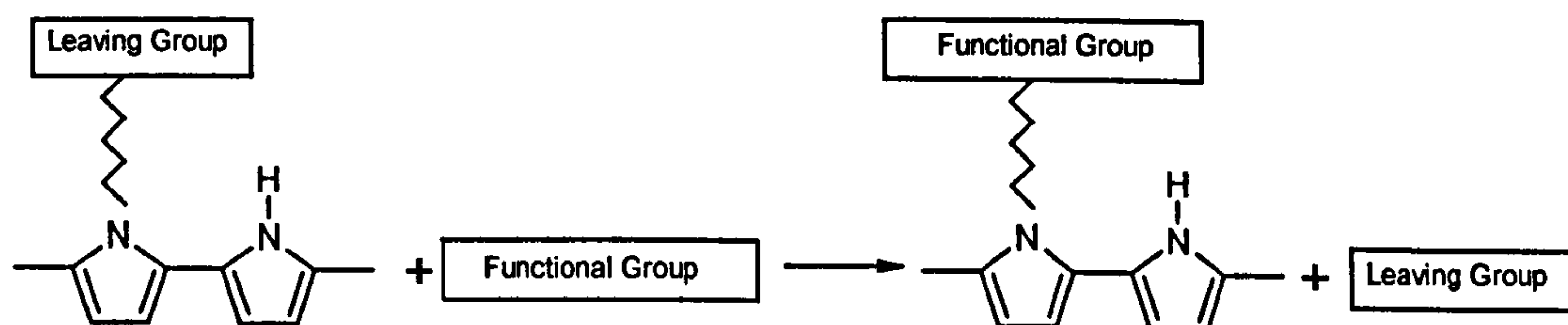
**Figure 1.3:** Diagram illustrating functionalised homopolymer and copolymer films, showing:- (a) a homopolymer (above); and (b) a copolymer arrangement (below).



**Figure 1.4:** Polymerisation of a functionalised pyrrole monomer with non-functionalised pyrrole monomer to form a functional copolymer film, forming a copolymeric form, similar to Figure 1.3 (b).

### 1.1.3.3 Polymerisation of Monomers with Activated Leaving Groups

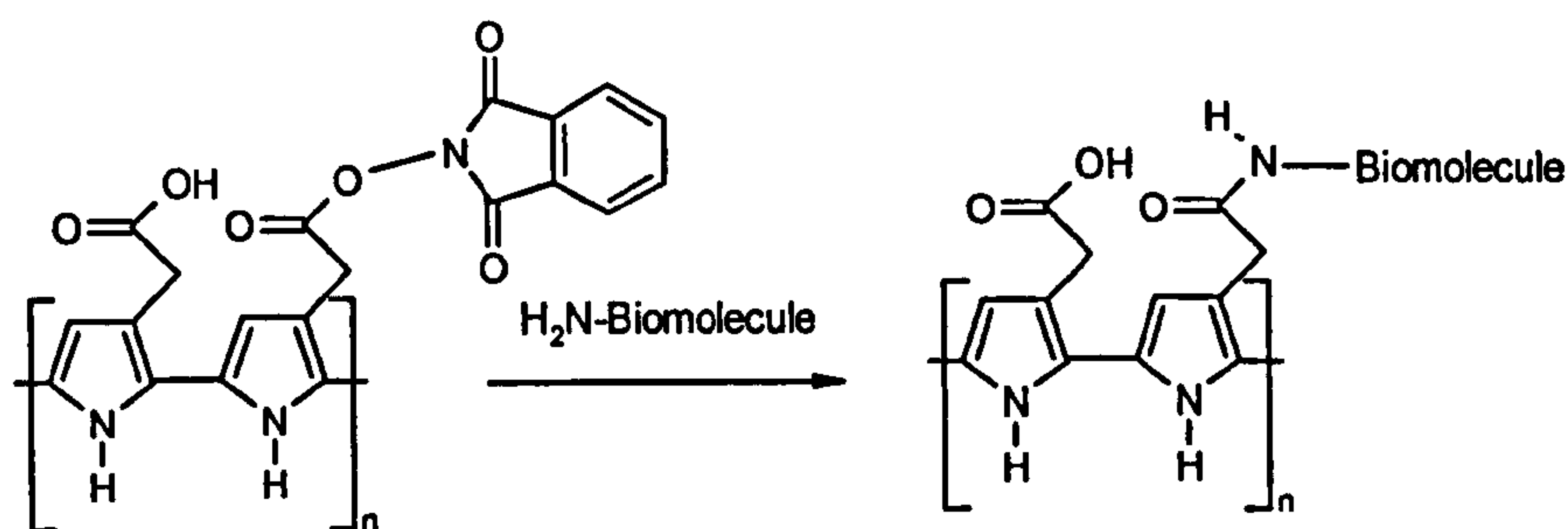
The final approach, which is an extension to the method used in section 1.1.3.1, is to synthesise electroactive, electropolymerisable monomers that can retain high electrical conductivity while possessing pendant leaving groups suitable for subsequent specific immobilisation. The formation an electroactive polymer, which contains activating leaving groups, may serve as the basis for the subsequent chemical reactions producing a biomolecule containing membrane.<sup>43, 45-46</sup>



**Figure 1.5:** Schematic diagram illustrating the reaction of a functional group with a preformed polymer film. This protocol represents the underlying technique used extensively within this thesis.

Previous work has illustrated the use of a substitution reaction for the immobilisation of functional groups. An example of which involves a precursor copolymer, poly[(3-acetic acid pyrrole)/(3-*N*-hydroxyphthalimide)], as seen in Figure 1.6. This precursor polymer allows a direct chemical substitution of this labile ester group with a group

bearing a terminal amino function. The biomolecules were “grafted on” by a chemical substitution of leaving group N-hydroxyphthalamide.

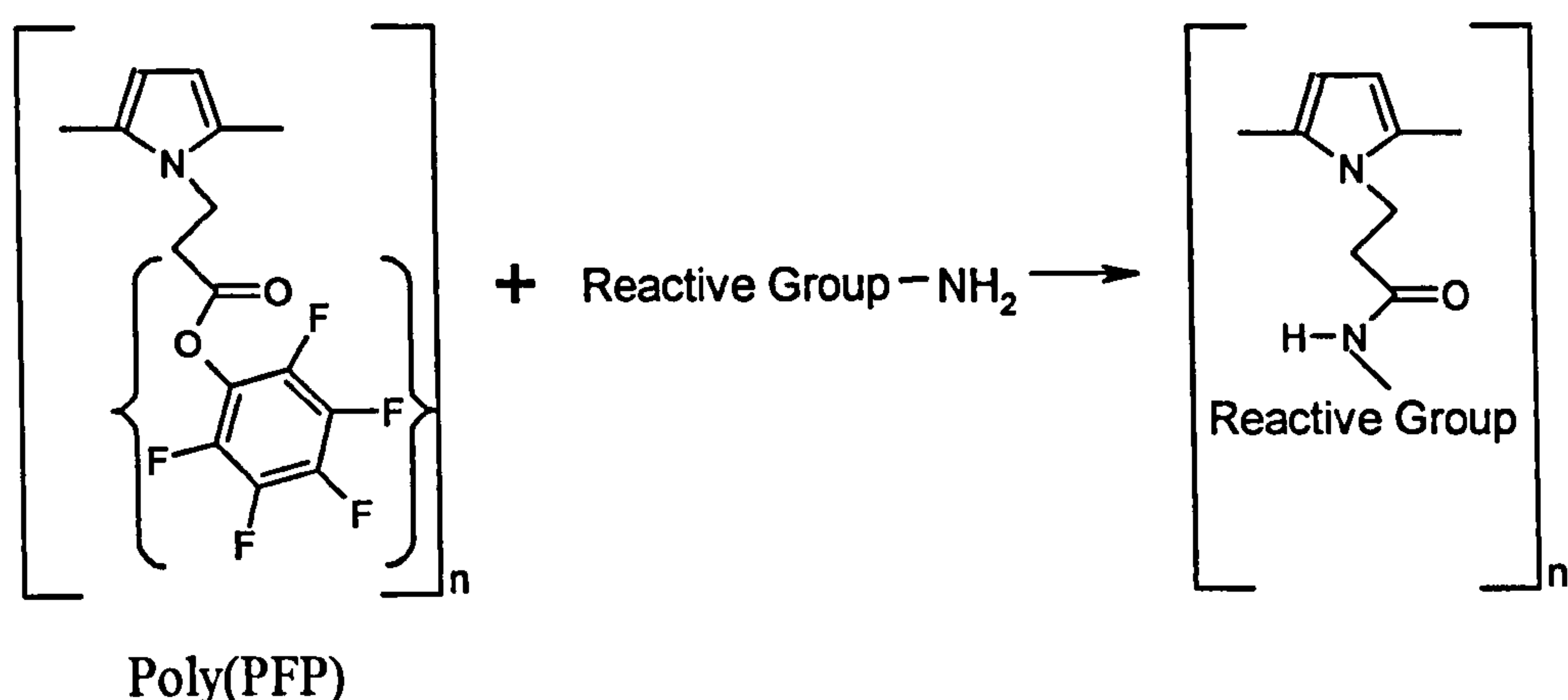


**Figure 1.6:** Reaction scheme of an amine terminated biomolecule with an activated ester present within a copolymer film.

The polymers used in the research presented in this thesis are homopolymers and copolymers developed using a pyrrole derivative, which contains an organic moiety that can be used for biomolecule immobilisation. The pyrrole derivative used, pentafluorophenyl 3-(pyrrol-1-) propanoate (PFP), bears an ester moiety activated by a “good” leaving group. This group is readily susceptible to nucleophilic attack (e.g. by amine groups often found on the outer surface of proteins, or on terminal groupings of strands of DNA) and thus the polymer provides reactive sites for the immobilisation of a desired species by covalent bond formation.<sup>47</sup>

Previous work had resulted in successful modification of PFP polymers of the activated ester. These modifications have involved substitution of the pentafluorophenolate ester with methanol to produce methyl ester and ferrocene methanol to produce a redox active ferrocenyl-modified polymer.





**Figure 1.7:** Schematic of the reaction of poly(PFP) with an amine terminated reactive group. The brackets seen show the activated ester.

#### 1.1.4 Patterning of Electrodes with Polymeric Adducts

Among the desirable attributes of using these of conducting polymers is the ability to place the polymer matrix in specific, electronically addressed, regions of a substrate such as a metallic micro-array. Since these electrodeposited conducting polymers can be used for the immobilisation matrix for various biospecies, the aim of this work is to attempt to immobilise different species in different locations on the same substrate.<sup>48</sup> By electrically controlling the nature of the polymerisation conditions, it is possible to precisely control the thickness of film, modifying the electrode.

Two methods used to pattern different species on the same substrate have been described. The first method reported involves sequentially depositing the polymer matrix in different locations must be carried out with intervening coupling to the appropriate species.<sup>49</sup> The second method that has been used involves polymer activation by light passing through spatially discrete locations in an optical mask.<sup>50</sup>

As with many techniques, both of these approaches have advantages and limitations: with the former (sequential polymer) deposition, the required deposition solution conditions may cause damage to the previously deposited species. Also the reactions

between reactive monomer units and the immobilised species can lead to a loss in effectiveness of the array sensor. With the latter, light activation of the polymer, the continual need for realigning of the optical mask (often within a solution), and the short-lived nature of the reactive species likewise limits the applicability of this approach. When the aim is to couple several different species to different locations on the same device, it is necessary to, develop a technique to allow micropatterning of different species of electrodes of the same device.

A new method is developed in this research, which utilises the simple use of electrochemical potential modulation to promote, or inhibit the coupling between a polymer bound activated ester group, of PFP homopolymer, and solution species containing nucleophilic amine groups. Examples are illustrating the control of the substitution reactions with various nucleophilic species are discussed further in Chapters 3, 4 and 5.

## ***1.2 Strategy of Bio-Polymeric Functionalisation***

Various strategies to allow the immobilisation of functional groups within the polymer film have been described. One technique, which involves direct reaction of the functional groups, present within the already prepared polymer film, is utilised during this research. This involves immersing the polymer-modified electrodes into a solution containing the desired nucleophilic species. Optimisation of this route of attachment requires careful study of both the polymerisation conditions and the chemical conditions necessary for the substitution of the functional group to take place. This is achieved by analysing the samples, using a variety of analytical techniques, to determine whether the immobilisation reactions have been successful. From these results both polymer preparation and modification condition can be altered to achieve optimum substitution reaction.

This thesis concentrates on the development of a polymer system that has ultimately been used for biomolecule immobilisation and, moreover, could be adapted for incorporation to into a miniaturised multi-analytical biosensor system.

### ***1.3 Outline of Thesis Structure***

The work presented in this thesis has been separated into seven further chapters:

Chapter 2 introduces the main techniques used for the polymerisation and analysis of unreacted and modified polymers, during this research. These techniques include electrochemical methods: i.e. cyclic voltammetry; potential step chronoamperometry and electrochemical quartz crystal microgravimetry. The chapter illustrates how these techniques are used for the synthesis and analysis of unmodified PFP homopolymer and PFP/Py copolymer films.

Chapter 3 describes the spectroscopic methods: X-ray photoelectron spectroscopy (XPS), XPS imaging and reflectance-adsorption infrared spectroscopy, which were used to analyse the polymers produced using the methods described in Chapter 2. It also describes the differences in polymer composition and morphology of individual films polymerised using these electrochemical techniques. The chapter also introduces the methods used to analyse poly(PFP) films after reaction with nucleophilic species.

Chapter 4 presents the results of the simple nucleophilic substitution reaction of polymer films with synthetic amine-containing species. The kinetics for each of the reactions with different homopolymer and copolymer films was investigated and the results are discussed.

Chapter 5 introduces a technique to inhibit the substitution reactions of the activated esters of the polymer films with amines is also introduced. This technique is then used for the micropatterning of the amines on interdigitated electrodes and images obtained from the XPS image facility to illustrate this are presented.

Chapter 6 presents results of reactions, which modify the PFP homopolymer matrices, to produce a multicomponent system. This involves nucleophilic reaction



with a biological amine cystamine, which is used to interact with a platinum complex. Examples are then illustrated of further immobilisation of amines with this complex, thus developing a system that could be potentially used for biomolecule immobilisation.

Chapter 7 exploits the use of biotinylated polymer films interaction with streptavidin and streptavidin-enzyme conjugates. It describes two methods of attaching biotin to the PFP homopolymer. The first involves a reaction with 5-(biotinamido) pentylamine solution then the subsequent reaction with streptavidin. The second involves the reaction of cystamine modified film with a biotin derivative then streptavidin. The latter method is useful as it allows the possible patterning of cystamine and, consequently, streptavidin species.

Chapter 8 describes a method to inhibit non-specific binding on a polymer surface. These preliminary investigations involve grafting poly (ethylene glycol) (of different molecular weights) onto the PFP homopolymer backbone. These modified polymers are then immersed in solutions containing labelled proteins to determine whether the prevention of non-specific binding is successful.

Chapter 9 provides the overall conclusion of the work performed and describes further work that is being carried out in the department, which relates to the research described in this thesis.

## ***1.4 References***

1. Sethi, R.S., "Transducer aspects of biosensor", *Biosensors and Bioelectronics*, vol. 9, no. 3, pp. 243-264, 1994.
2. Nicolini, C., "From neural chip and engineered biomolecules to bioelectronic devices: An overview", *Biosensors and Bioelectronics*, vol. 10, pp. 105-127, 1995.
3. Aizawa, M., "Biosensors for molecular identification, in *Intelligent Sensors*", H. Yamasaki, Elsevier Science, pp. 85-97, 1996.



4. Aizawa, M., "Molecular Interfacing for Protein Molecular Devices and Neurodevices", *IEEE Engineering in Medicine and Biology*, February/March, pp. 94-102, 1994.
5. Cooper, J.M. and C.J. McNeil, "Biosensors", in "The Encyclopaedia of Advanced Materials", D. Bloor, et al., Pergamon, pp. 257-264, 1994.
6. Ghindilis, A.L., Atanaso, P., Wilkins, M., Wilkins, E., "Immunosensors: Electrochemical Sensing and Other Engineering Approaches", *Biosensors and Bioelectronics*, vol. 13, no. 1, pp. 113, 1998.
7. Giuliano, K.A. and D.L. Taylor, "Fluorescent-Protein Biosensors: New Tools for Drug Discovery", *Trends in Biotechnology*, vol. 16, no. 3, pp. 135, 1998.
8. Hellinga, H.W and J.S. Marvin, "Protein Engineering and the development of generic biosensors", *Trends in Biotechnology*, vol. 16, no. 4, pp. 183, 1998.
9. Byfield, M.P. and R.A. Abukeshna, "Biochemical aspects of biosensors", *G.E.C.J. of Research*, vol. 9, no. 2, pp97, 1991.
10. Holden Thorp, H., "Cutting out the Middleman: DNA Biosensors Based on Electrochemical Oxidation", *Trends in Biotechnology*, vol. 16, no. 3, pp. 117, 1998.
11. Skotheim, T. A., Elsenbauner, R. L., Reynolds, J. R., "Chemical and Biological Sensors Based on Electrically Conducting Polymers" in, "Handbook of Conducting Polymers", Mercel Dekker, New York, 1998.
12. Hall, E. A. H., "Biosensors", Open University Press, Milton Keynes, 1990 .
13. Anderson, G.P., "Development of an evanescent wave fiber optic biosensor", *IEEE Engineering in Medicine and Biology*, pp. 358, 1994.

14. Barker, S.L.R, et al., "Fiber-Optic Nitric Oxide-Selective Biosensors and Nanosensors", *Analytical Chemistry*, vol. 70, no. 5, pp. 971, 1998.
15. Bond, A.M., "Past, present and future contributions of microelectrodes to analytical studies employing voltammetric detection", *Analyst*, vol. 119, pp. R1-R21, 1994.
16. Bruckenstein, S. and M. Shay, "Experimental aspects of use of the quartz crystal microbalance in solution", *Electrochimica Acta*, vol. 30, no. 10, pp. 1295, 1985.
17. Keese, C.R. and I. Giaever, "A biosensor that monitors cell morphology with electric fields", *IEEE Engineering in Medicine and Biology*, pp. 402, 1994.
18. Muehlbauer, M.J., Guilbeau, E. J., Towe, B. C., Brandon, T. A., "Thermoelectric enzyme sensor for measuring blood glucose", *Biosensors and Bioelectronics*, vol. 5, pp. 1, 1990.
19. Nabauer, A., Berg, P., Ruge, I., Muller, E., Woiass, P., Kosslinger, C., Drobe, H., "Biosensors based on piezoelectric crystals", *Sensors and Actuators B*, vol. 1, pp. 508, 1990.
20. Soller, B.R., "Design of intravascular fiber optic blood gas sensors", *IEEE Engineering in Medicine and Biology*, pp. 327, 1994.
21. Turner, A.P.F., I. Karube, and G.S. Wilson, "Biosensors: Fundamentals and Applications", 1st ed., Oxford: Oxford University Press, pp. 747, 1987.
22. Livache, T., Bazin, H., Mathis, G., "Conducting polymers on microelectronic devices as tools for biological analyses", *Clinica Chimica Acta*, vol. 278, pp. 171, 1998.
23. Yegnaraman, V., Yacynych, M., "Enzyme Immobilisation on Ultramicroelectrodes through electropolymerisation: Effect of polymeric film

thickness on the amperometric response of the electrode”, *Bulletin of Electrochemistry*, vol. 16, no. 1, pp. 21, 2000.

24. Wallace, G. G., Smyth, M., Zhao, H., “Conducting electroactive polymer-based biosensors”, *Trends in Analytical Chemistry*, vol. 18, no. 4, pp. 245, 1999.

25. Cosnier, S., “Fabrication of Amperometric Biosensors by Entrapment of Enzymes in Functionalised Polypyrrole Films”, *The Canadian Journal of Chemical Engineering*, vol. 76, pp. 1000, 1998.

26. Bidan, G., Billon, M., Livache, T., Roget, A., Torres-Rodriguez, L. M., “Conducting polymers as a link between biomolecules and microelectronics”, *Synthetic Metals*, vol. 102, pp. 1363, 1999.

27. Ryder, K. S., Morris, D. G., Cooper, J. M., “Functionalisation and Characterisation of Novel Conducting Polymer Interfaces”, *Journal of Chemical Society, Chemical Communication*, pp. 1471, 1995.

28. Cooper, J. M., Morris, D. G., Ryder, K. S., “A Bio-electronic Interface using Functionalised Conducting Poly(pyrroles)”, *Journal of Chemical Society, Chemical Communication*, pp. 697, 1995.

29. Hoshi, T., Anzai, J., Osa, T., “Controlled deposition of glucose oxidase on platinum electrode based on an avidin/biotin system for the regulation of output current of glucose sensors”, *Analytical Chemistry*, vol. 67, pp. 770, 1995.

30. Davis, J., Glidle, A., Cass, A. E. G., Zhang, J., Cooper, J. M., “Spectroscopic Evaluation of Protein Affinity Binding at Polymeric Biosensor Films”, *Journal of American Chemical Society*, vol. 121, pp. 4302, 1999.

31. Gerard, M., Chaubey, A., Malhorta, B. D., “Application of conducting polymers to biosensors”, *Biosensors and Bioelectronics*, vol. 17, pp. 349, 2002.

32. Gardner, J. W., Bartlett, P. N., "Application of conducting polymer technology in microsystems", *Sensors and Actuators A*, vol. 51, pp. 57, 1995.
33. Bartlett, P. N., Cooper, J. M., "A review of the immobilisation of enzymes in electropolymerised films", *Electroanalytical Chemistry*, vol. 1993. 362: p1
34. Kranz, C., Wohlschlager, H., Schmidt, H-L., Schuhmann, W., "Controlled Electrochemical Preparation of Amperometric Biosensors Based on Conducting Polymer Multilayers", *Electroanalysis*, vol. 10, pp. 546, 1998.
35. Genies, E. M., Bidan, G., Diaz, A. F., "Spectroelectrochemical study of polypyrrole films", *Journal of Electroanalytical Chemistry*, vol. 149, pp. 101, 1987.
36. Cosnier, S., "Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films", *Biosensors and Bioelectronics*, vol. 14, pp. 443, 1999.
37. Korri-Yousoufi, H., Godillot, P., Srivastava, P., El Kassmi, A., Garnier, F., "New method of polypyrrole Functionalization Toward Molecular Recognition", *Synthetic Metals*, vol. 84, pp. 169, 1997.
38. Godillot, P., Korri-Yousoufi, H., Srivastava, P., El Kassmi, A., "Garnier, F., Direct chemical functionalization of as-grown electroactive polypyrrole film containing leaving groups", 1996. 83: p 117.
39. Heller, A., Kranz, C., Huber, J., Bauerle, P., Schuhmann, W., "Amperometric biosensors produced by immobilisation of redox enzymes at polythiophene-modified electrode surfaces", *Advanced Materials*, vol. 8, no. 3, pp. 219, 1996.
40. Wolowacz, S. E., Yon-Hin, B. F. Y., Lowe, C. R., "Covalent immobilisation of glucose oxidase in poly-pyrrole", *Analytical Chemistry*, vol. 64, no. 14, pp. 1541, 1992.



41. Yon-Hin, B. F. Y., Smolander, M., Crompton, T., Lowe, C. R., "Covalent electropolymerisation of glucose oxidase in pyrrole: evaluation of methods of pyrrole attachment to glucose oxidase on the performance of electropolymerised glucose sensors", *Analytical Chemistry*, vol. 65, pp. 2067, 1993.
42. Rosenthal, M. V., Skotheim, T. A., Linkous, C. A., "Polypyrrole-phthalocyanine", *Synthetic Metals*, vol. 15, pp. 219, 1986.
43. Guiseppi-Elie, A., Wilson, A. M., "Electroconductive polymer thin films with internal bioactivity for biosensors", *Proc. ACS div. Polymer Material Science Engineering*, vol. 72, pp. 404, 1995.
44. Roncali, J., Korri, H., Garreu, R., Garnier, F., Lemaire, M., "New Electrogenated Conducting Poly(thiophenes) Containing Substituted Phenyl Groups", *Journal of Chemical Society, Chemical Communication*, pp. 414, 1990.
45. Guiseppi-Elie, A., Wilson, A. M., Tour, J. M., Brockmann, T. W., Zhang, P., Allara, D. I., "Specific immobilisation of electropolymerised polypyrrole thin films onto interdigitated microsensor electrode arrays", *Langmuir*, vol. 11, pp. 1768, 1995.
46. Cooper, J. M., Morris, D. G., Ryder, K. S., "A bioelectronic interface using functionalised conducting poly(pyrroles)", *Journal of Chemical Society, Chemical Communication*, pp. 697, 1995.
47. Pickett, C. J., Ryder, K. S., "Bioinorganic Reaction Centres on Electrodes. Modified Electrodes possessing Amino Acid, Peptide and Ferredoxin-type groups on a poly(pyrrole) Backbone", *Journal of Chemical Society, Dalton Transaction*, pp. 2181, 1994.
48. Garnier, F., KorriYousoufi, H., Srivastava, P., Mandrand, B., Delair, T., "Toward intelligent polymer: DNA sensors based on oligonucleotide functionalized polypyrroles", *Synthetic Metals*, vol. 100, pp. 89, 1999.

49. Rozsnyai, L., Wrighton, M., "Selective Deposition of Conducting Polymers via monolayer photopatterning", *Langmuir*, vol. 11, pp. 3913, 1995.
50. Britland, F., Clark, P., Connolly, P., Moores, G., "Micropatterned Substratum Adhesiveness- A model for morphocenetic cues controlling behaviour", *Exp. Cell Research*, vol. 198, pp. 124, 1992.

# Chapter 2

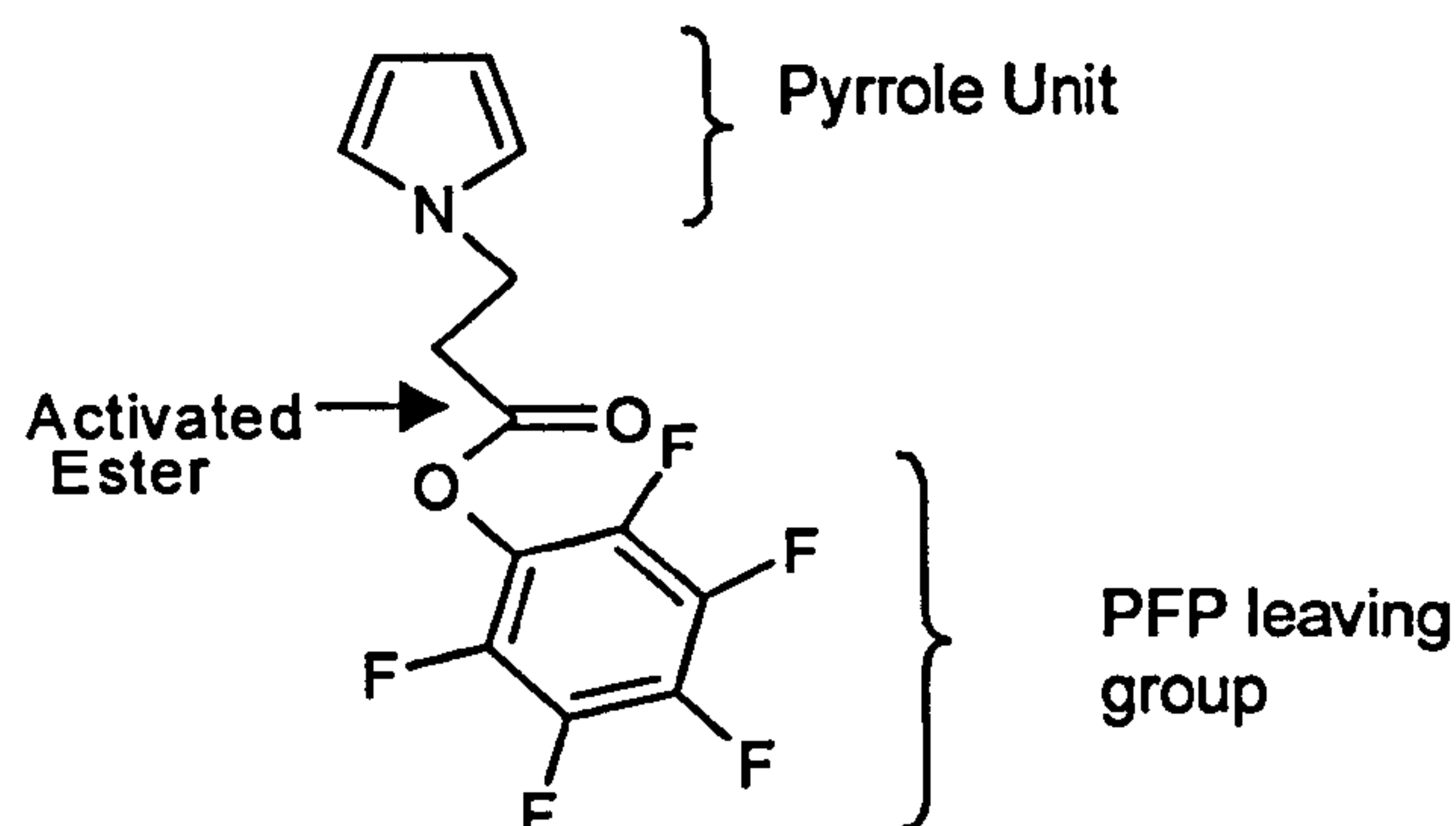
## *Electrochemical Methods used for the Development and Characterisation of a Polymer System*

### *2.0 Introduction*

In this chapter a brief description is given of the techniques used in the characterisation and development of the modified polymer electrodes used in this thesis. These techniques are illustrated using examples of measurements obtained from the polymerisation and analysis of the polymers, used during this research. An in depth discussion of the application of different modifications and characterisation of these polymers is given in later chapters.

The various functionalised polymers developed were synthesised using pyrrole and/or pyrrole derivatives. The substrate bound polymer films were prepared by electrochemical oxidation (or electropolymerisation) of an appropriate monomer at metal electrodes. This can be achieved either through scanning (cyclic voltammetry) or stepping (potential step chronoamperometry) the potential of the “working” electrode whilst immersed in the monomer containing deposition solution. In both cases the electrode potential must be sufficient to oxidise the monomer species, although it was noted that, the use of a scanned potential program offers greater control over polymer deposition rates, internal microstructure and final film thickness.

In these investigations, the majority of functionalised conducting electropolymerised thin films were based on the pyrrole derivative, pentafluorophenyl 3- (pyrrol-1-yl) propionate (PFP) moiety (Figure 2.1). This provides a model system in which the electron withdrawing properties of the pentafluorophenyl group activates the ester moiety and, therefore, enables a suitable reactive site for the coupling of a nucleophilic species (e.g. primary or secondary amine groups).



**Figure 2.1:** Illustration of PFP monomer unit indicating the important features; pyrrole unit, activated ester and pentafluorophenyl (PFP) leaving group.

A further advantage of the use of this system is that it contains strong spectroscopic features, the pentafluorophenol group, which can be quantitatively analysed using XPS, and the ester group, which can be identified in the bulk polymeric structure, using FTIR.

## 2.1 Materials

**Solvents:** Dichloromethane (99.9%, HPLC grade), acetonitrile (MeCN, 99.5+%, ACS reagent, CH<sub>3</sub>CN), dimethylsulphoxide (DMSO, methyl sulphoxide, 99+%, anhydrous, (CH<sub>3</sub>)<sub>2</sub>SO), acetone (99.5+%, ACS spectroscopic grade, CH<sub>3</sub>COCH<sub>3</sub>), dioxane (1, 4-Dioxane, 99+%, spectrophotometric grade), iso-propyl alcohol (IPA, 2-Propanol, 99.5+%, ACS spectrophotometric grade, (CH<sub>3</sub>)<sub>2</sub>CHOH) and diethyl ether (99+%, ACS reagent) were obtained from Aldrich.



Other materials were used to prepare the following:

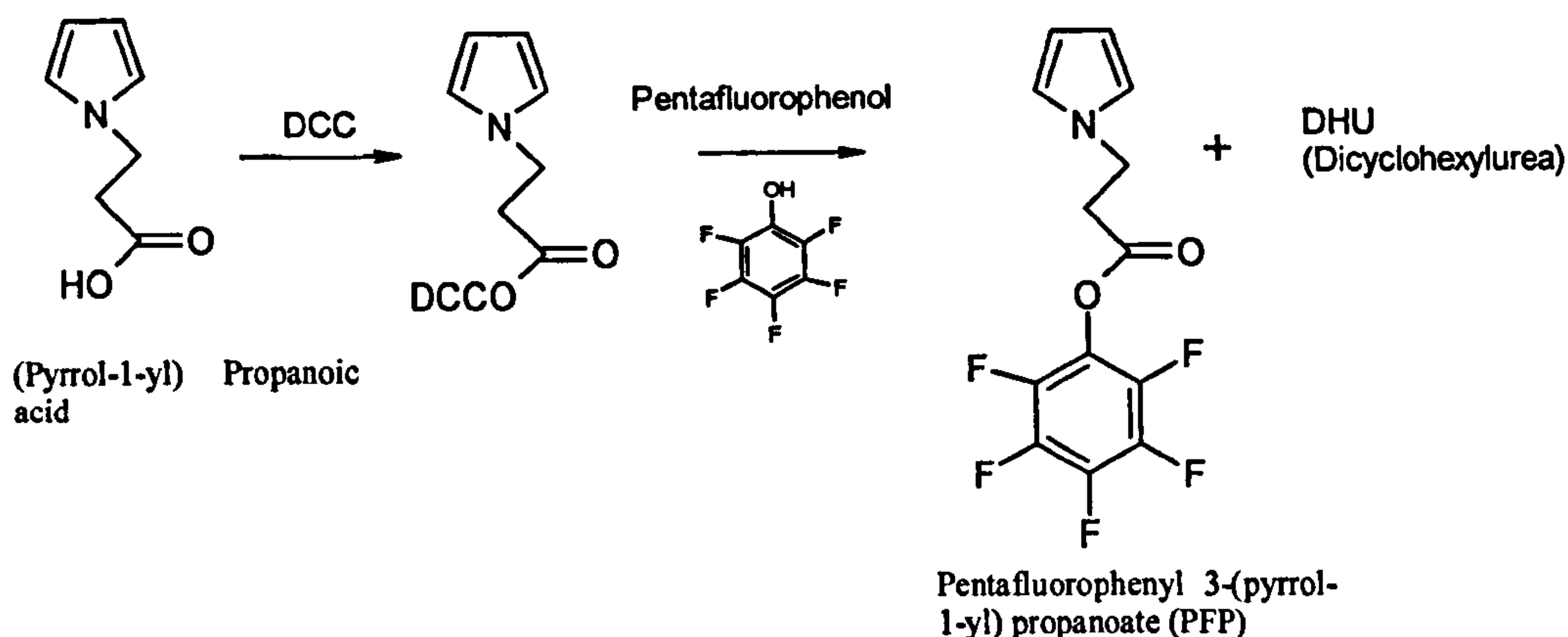
**Synthesis of 3-(pyrrol-1-yl) propanoic acid (PyCOOH)<sup>1</sup>:**

12g of 3-(pyrrol-1-yl) propionitrile (obtained from Sigma-Aldrich) was added to 100 ml of 50% sodium hydroxide (pellets, 97+%, ACS reagent, obtained from Aldrich) and refluxed until no ammonia was released. 50 % H<sub>2</sub>SO<sub>4</sub> (95-98 % obtained from Aldrich) was added until the solution was pH 2. The mixture was then extracted using ether (Aldrich). The extracts were combined and evaporated to produce 3-(pyrrol-1-yl) propanoic acid with yield of 87%.

A widely used method to esterify a carboxylic acid is to treat it with an alcohol in the presence of a dehydrating agent. One such dehydrating agent is dicyclohexylcarbodiimide (DCC), which is subsequently converted to dicyclohexylurea (DHU). Using this method PyCOOH is converted to a compound with a better leaving group, which allows reaction with pentafluorophenol, and which can be probed in the polymeric form spectroscopically.

**Synthesis of Pentafluorophenyl 3-(pyrrol-1-yl) propanoate (PFP):**

6g of (Pyrrol-1-yl) propanoic acid (PyCOOH) was added to 100 ml of dioxane and stirred. 8g pentafluorophenol (99+% obtained from Aldrich) was then added along with 9.06g dicyclohexylcarbodiimide (1,3-Dicyclohexylcarbodiimide, 99% obtained from Aldrich)). A precipitate developed after stirring overnight, which was filtered off using a vacuum pump. The solvent was removed under vacuum to produce a crude oil. The crude oil was run down a silica (Aldrich) column using 20% dichloromethane and 80% petrol to yield PFP.



**Figure 2.2:** Reaction scheme of the synthesis of PFP from (Pyrrol-1-yl) Propanoic Acid.

### Preparation of Tetraethylammonium Perchlorate Electrolyte (TEAP):

50 ml of 20% w/w Tetraethylammonium hydroxide (1.3 M, Aldrich) was poured into a 250ml conical flask (approximately 0.067 moles). This was then placed in a cooling bath (ice/water) and was stirred with a magnetic stirrer. 6 ml of 70% w/w perchloric acid (11M, Aldrich) was placed in a 25ml conical flask and was similarly cooled (0.067 moles). Once both solutions were cooled, perchloric acid was added dropwise to the hydroxide solution. During the addition a white precipitate was formed. This was recrystallised from water several times to produce tetraethylammonium perchlorate. This was dried and stored in a desiccator until further use.

### Pyrrole purification:

Pyrrole (98%, Aldrich) was purified by neutral alumina column (neutral aluminium oxide powder, Aldrich) chromatography before using it in the deposition solution for polymerisation. This involved running the crude liquid down a short column (3 cm x 0.5 cm<sup>2</sup>) filled with neutral aluminium oxide to remove oligomers (dark brown material), present in the pyrrole solution.

### Protocol for Electrode Fabrication<sup>2</sup>:

The protocol of electrode fabrication is shown in Figure 2.3 from (A) to (F). First, microscope glass slides were cleaned by sonicating using Decon and were thoroughly washed with copious amounts of water. They were then chemically modified by silanisation to generate a surface with free S-H groups (using a thiol

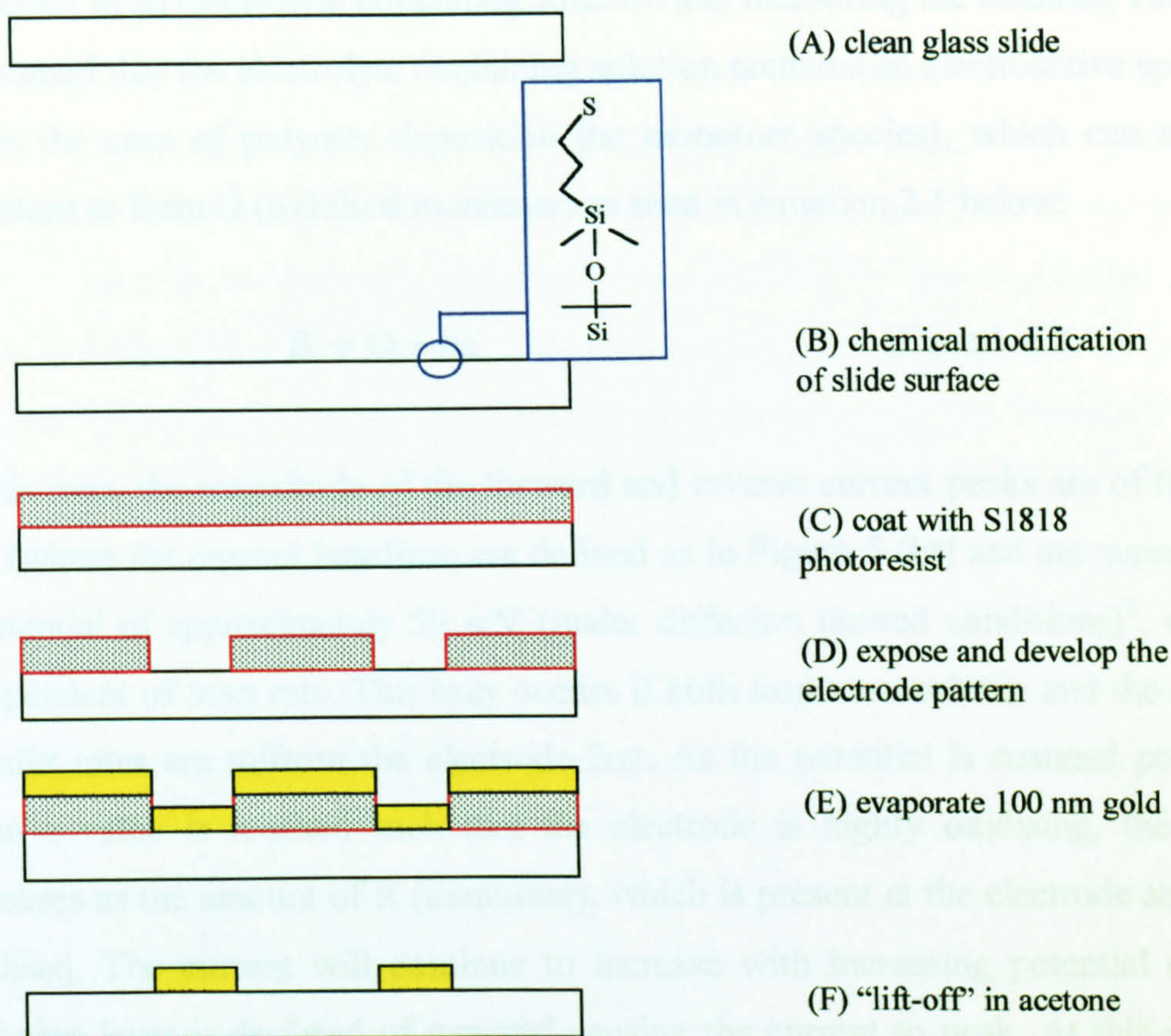
solution of 3-mercaptopropyl trimethoxy silane (“silane), obtained from Aldrich). The protocol involved refluxing the slides (in a round bottom flask with condenser attached) in a 20 mM solution of silane for approximately 1 hour (solution was made up of 12g silane and 12g water in 400 ml of IPA). This was repeated to ensure that there is an even molecular layer with sulphur functionalities, which will bind to the evaporated gold via strong thiol bonds. The slides were then loaded into the gold metal evaporator. Once a pressure of  $4 \times 10^{-6}$  Torr had been reached in the evaporation chamber, 100 nm of gold was deposited using the Balzers PKR 250 thin film deposition system. The thickness of Au was controlled by an Intellemetrics IL 150 programmable interface (the thickness was determined by difference of frequencies of quartz crystals).

To produce patterned electrodes that were used for research described in future chapters, the thiolated slides were coated with S1818 photoresist (Shipley Ltd, UK) by spinning at 4000 rpm for 30 seconds and baking at 90°C for 30 minutes, giving a resist thickness of 1.8  $\mu\text{m}$ . The resist coated slides were exposed to UV light through a chrome mask for 12 seconds on a mask aligner and developed with AZ developer (Shipley Ltd, UK) for 90 seconds. After gold deposition, and as before, the pattern of the electrodes was developed by “lift-off” by immersing the slides in acetone. Once the unnecessary gold was removed the slides were examined under a microscope to ensure the patterning was successful (Figure 2.3 (F)).

## ***2.2 Electrochemical Methods***

In using cyclic voltammetry and potential step chronoamperometry methods, it is possible to measure the electrode current as a function potential and/or time and thereby control the rate and extent of polymer deposition<sup>3</sup>. These techniques were mainly employed to polymerise homopolymer and copolymer modified electrodes. Another electrochemical method used in these investigations was quartz crystal microgravimetry, which provides a technique that can determine the mass changes of the working electrode during polymerisation and during immobilisation reactions<sup>3</sup>. This section provides an overview of the principles of each of these methods.





**Figure 2.3:** Protocol for the development electrodes subsequently used for the polymerisation of homopolymer and copolymers. A variety of different methods exist to produce the patterned electrodes, based upon photolithography of either positive or negative photoresists, lift-off and/or etching.

## 2.2.1 Cyclic Voltammetry

### 2.2.1.1 Background

Cyclic voltammetry is a commonly used technique, which is particularly powerful for the initial studies of a new system. Many experiments can be carried out within a few minutes. Moreover, the data are presented in a form, which allows rapid, qualitative interpretation without recourse to detailed calculation.



Cyclic voltammetry (CV) consists of scanning the potential of an electrode which is immersed in an electrolyte containing solution and measuring the resulting current. It is assumed that the electrolyte containing solution contains an electroactive species R (or in the case of polymer deposition the monomer species), which can undergo oxidation to form O (oxidised monomer) as seen in equation 2.1 below:



In this case, the magnitude of the forward and reverse current peaks are of the same size (where the current baselines are defined as in Figure 5 (b)) and are separated by a potential of approximately 59 mV (under diffusion limited conditions)<sup>4</sup>, which is independent of scan rate. This only occurs if both forms are soluble and the electron transfer rates are to/from the electrode fast. As the potential is scanned positively, when a value is reached such that the electrode is highly oxidising, the current increases as the amount of R (monomer), which is present at the electrode surface, is oxidised. The current will continue to increase with increasing potential until the diffusion layer is depleted of material causing the current to peak. At this point the current starts to decay as the thickness of the diffusion layer for R increases. Then, when the potential is scanned in a negative direction, the current slowly increases (negatively) until the potential is sufficiently reducing for the layer of O, which is produced on the forward scan and has accumulated at the surface. This continues with decreasing potential until the diffusion layer has been reduced, At this time a peak in the reduction current is observed, during this time a proportion of O diffuses into the bulk solution and is never reduced. Once again as the diffusion layer for O thickens with a build-up of R again, the current falls until the initial potential is reached and the scanning stops. The surface concentration of O will change according to the Nernst Equation:

$$[\text{O}]/[\text{R}] = \exp [nF/RT (E - E^0)] \quad (\text{Equation 2.2})$$

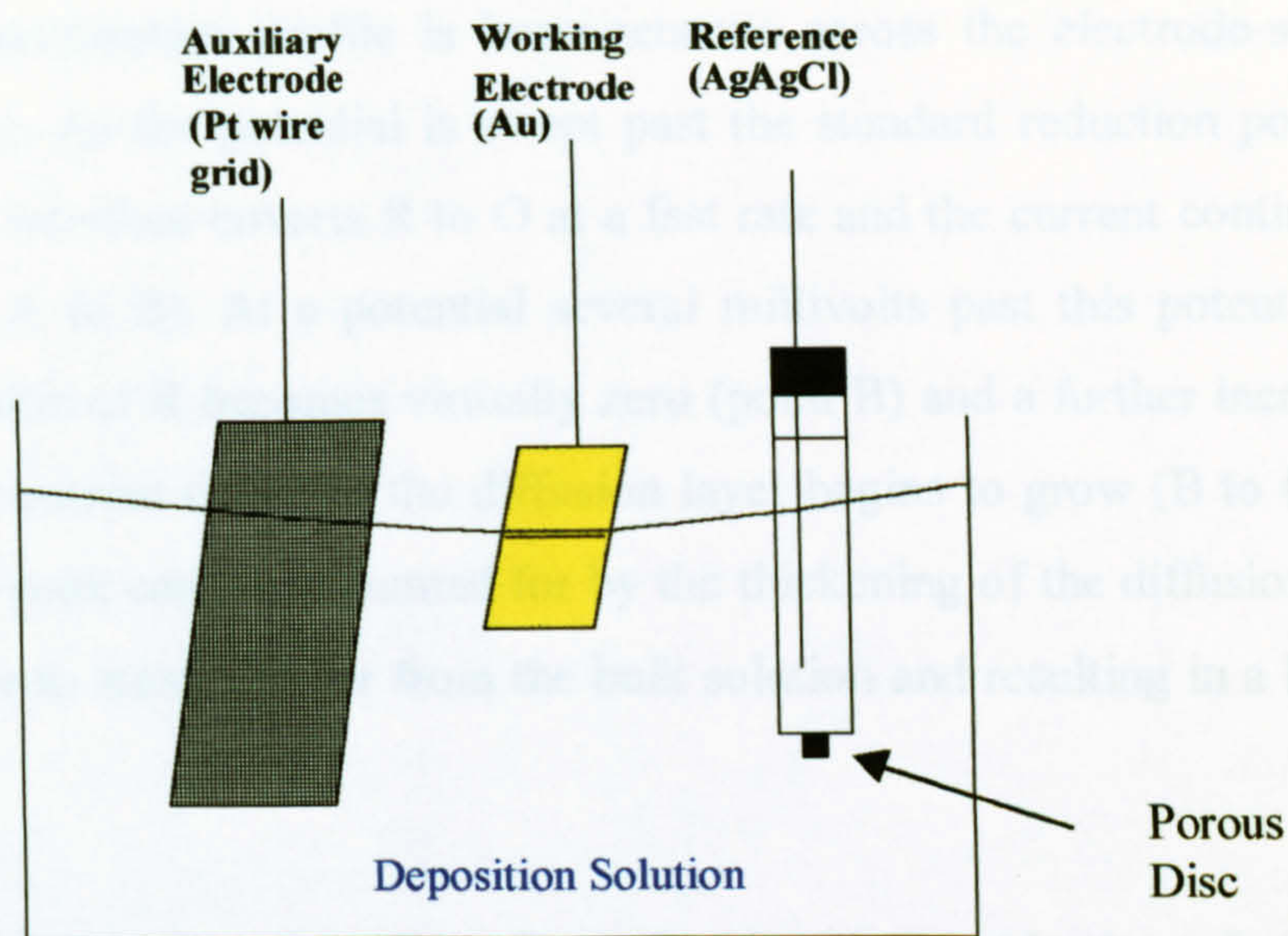
where  $E^0$  is the standard redox potential,  $E$  is the equilibrium potential,  $R$  is the molar gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ),  $F$  is the Faraday constant,  $n$  is the number of electrons transferred in the electrode reaction,  $[\text{O}]$  and  $[\text{R}]$  is the concentration of oxidised and reduced species respectively.

### 2.2.1.2 Instrumentation

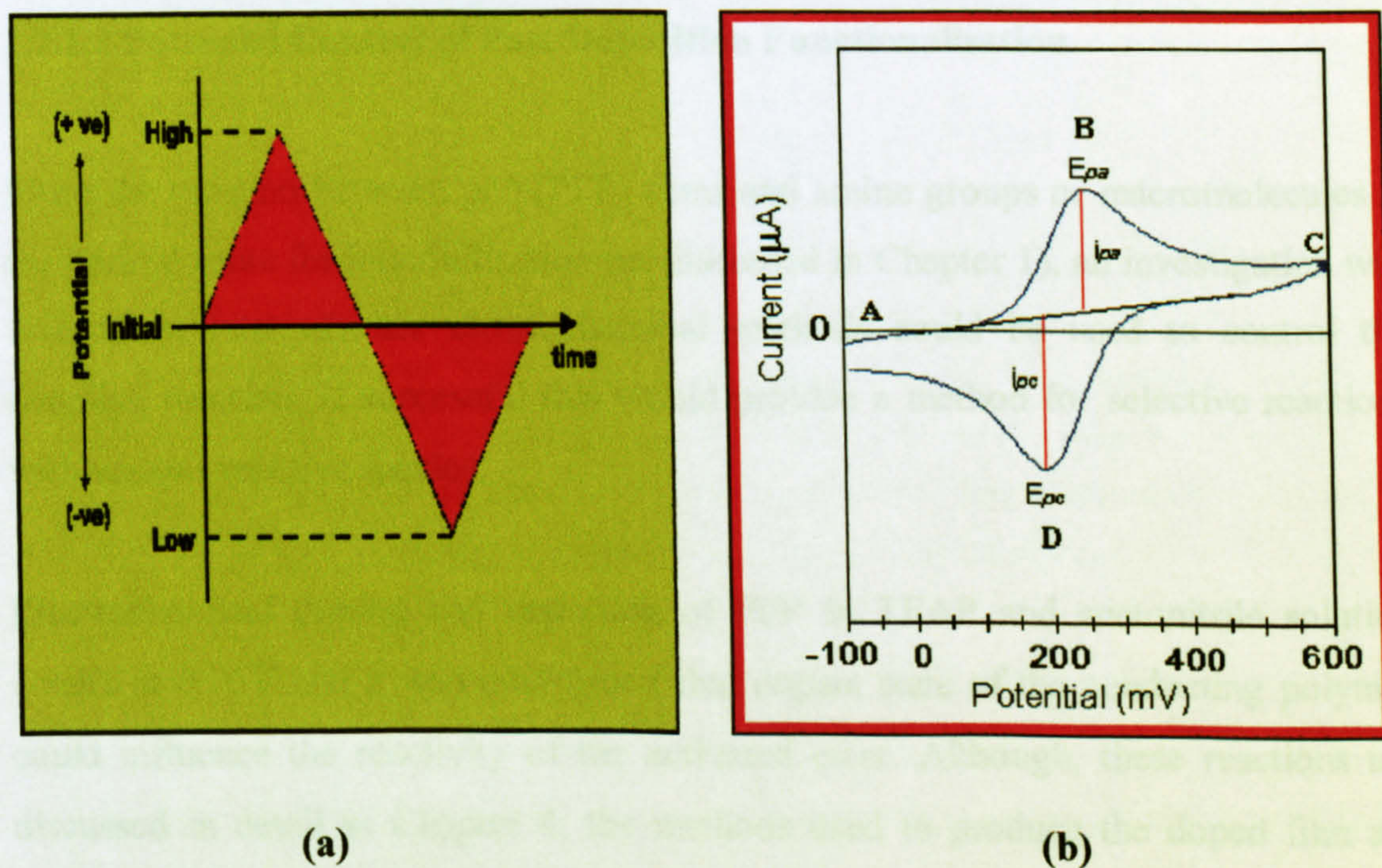
The experimental set-up used can be seen in Figure 2.4, and involves using a three-electrode system held in a cell containing the electroactive solution. The potential of the working electrode is controlled using a reference electrode (in these studies a silver/ silver chloride electrode ( $\text{Ag}|\text{AgCl}$ )/3M KCl). For the potential of the cell as a whole to reflect the potential of interest, it is necessary to keep the other half-cell (i.e. reference electrode) constant. The porous division forming the boundaries at the liquid junction provides good electrical contact between the KCl solution and the deposition solution, but also prevents a major interchange of the two liquids.

The reference electrode provides a stable fixed potential so that when a voltage is applied between the two electrodes the change in potential is precisely defined. The technique of cyclic voltammetry is related to Linear Sweep Voltammetry where the potential is only scanned in one direction<sup>4-6</sup>. In contrast, in CV, the potential of the working electrode is scanned between two values, firstly in the positive direction and then in the direction of the sweep is reversed and the electrode potential is scanned back to the original value. Single or multiple cycles can be used which at a constant scan rate are represented by a triangular potential cycle. This is illustrated in Figure 2.5 along with the typical cyclic voltammogram for a reversible couple one electron transfer.





**Figure 2.4:** Schematic diagram of the electrochemical cell set-up used for the polymerisation of the conducting polymers used in this research. The set-up includes a platinum counter electrode, gold working electrode and Ag|AgCl reference electrode.



**Figure 2.5:** (a) shows the variation of the applied potential as a function of time during a CV experiment, and (b) shows a cyclic voltammogram for a reversible electron transfer reaction<sup>7</sup>.



By observing Figure 2.5 (b), it can be noticed that at a resting potential, no current flows and the concentration profile is homogeneous across the electrode-solution interface (point A). As the potential is swept past the standard reduction potential, electrolysis at the interface converts R to O at a fast rate and the current continues to rise (from points A to B). At a potential several millivolts past this potential, the surface concentration of R becomes virtually zero (point B) and a further increase in potential causes a current decay as the diffusion layer begins to grow (B to C). The appearance of the peak can be accounted for by the thickening of the diffusion layer, providing a barrier to mass transfer from the bulk solution and resulting in a limiting current.

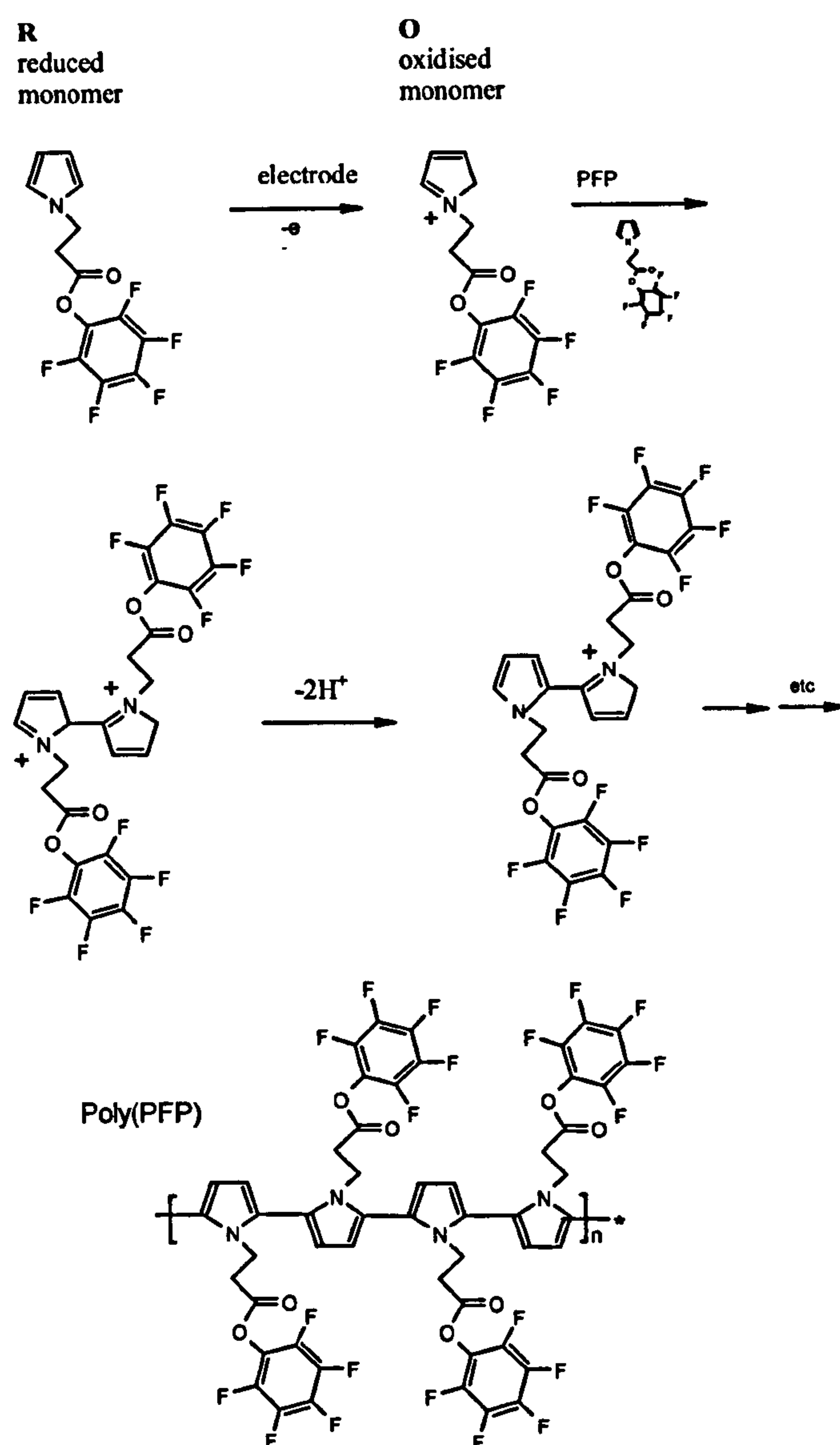
Cyclic Voltammetry can be applied to electrochemical polymerisation of particular monomer species, an example of which is pyrrole and pyrrole derivatives, such as PFP<sup>3,8</sup>. In the majority of the work in this thesis, the oxidation is carried out starting with reduced PFP monomer species (R) only and no oxidised species (O) initially present (see Figure 2.6).

### **2.2.1.3 Potential Control of Post Deposition Functionalisation**

Since the reaction between poly(PFP) films and amine groups or macromolecules is the desired route for immobilisation (as discussed in Chapter 1), an investigation was undertaken into whether electrochemical methods could be used to control the coupling reaction. If successful this would provide a method for selective reactions with various reactive species.

Electrochemical doping and undoping of PFP in TEAP and acetonitrile solution occurs at 0.76V and it was established that dopant state of the conducting polymer could influence the reactivity of the activated ester. Although, these reactions are discussed in detail in Chapter 4, the methods used to produce the doped film are outlined below.





**Figure 2.6:** Mechanism of electrochemical polymerisation of PFP monomer (using the electrochemical set-up detailed in Figure 2.4). As the voltage is increased oxidation of the monomers occurs at the electrode surface and these react to produce oligomers. This is followed by nucleation on the electrode surface. As the voltage is decreased the monomers are then reduced.

A homopolymer-modified Au electrode was placed in an acetonitrile solution containing 0.1 M TEAP as the supporting electrolyte. The electrode was cycled to an oxidising potential of approximately 0.77V and was held at this potential whilst being removed from the TEAP solution and then placed in an electrolyte solution containing a solution of the desired amine. The 0.77 V potential control was subsequently restored immediately on re-immersion. After a specified time, the

electrodes were again placed in an electrolyte solution that has no amine present, and the potential sweep was reversed to reduce the film at 0V. For direct comparison, another polymer- modified electrode was held at a potential of 0V in the particular amine solution for the same time interval. In addition, the homopolymer-modified electrode was also held in an amine free solution at 0.77V to determine whether there was any resultant change in the polymer film arising from the potential control above.

Further investigations were also carried out to establish whether nucleophilic reactions were inhibited if there was no potential control applied to the doped polymer during immersion in the amine solution. In these experiments the polymer was doped at 0.77V then the polymer electrode was left at open circuit during immersion in the designated amine solution. The results of the electrochemical and spectroscopic analysis performed to determine whether the reaction had taken place and are presented in Chapter 4.

## **2.2.2 Potential Step Chronoamperometry**

### **2.2.2.1 Background**

The potential of the working electrode is stepped between E1 and E2 as seen in Figure 2.7. The initial potential should be such that the species of interest is not oxidised/reduced so that when the potential is stepped to a value where oxidation/reduction is known to occur, a current transient is recorded that reflects the electron transfer reaction at the electrode surface. Immediately following the step, a large current is detected which falls steadily with time. This arises since the magnitude of the current is controlled by the rate of diffusion of R to the electrode. The concentration gradients shortly after the step are extremely large since there has been little time for any depletion of electroactive material. Consequently, the currents flowing are very large initially. Gradually depletion occurs as the diffusion layer thickness increases and the current decreases, ultimately to zero as shown in Figure 2.8. The Cottrell equation, Equation 2.3, describes the current response as a function of time (and electrode area)<sup>6</sup>.

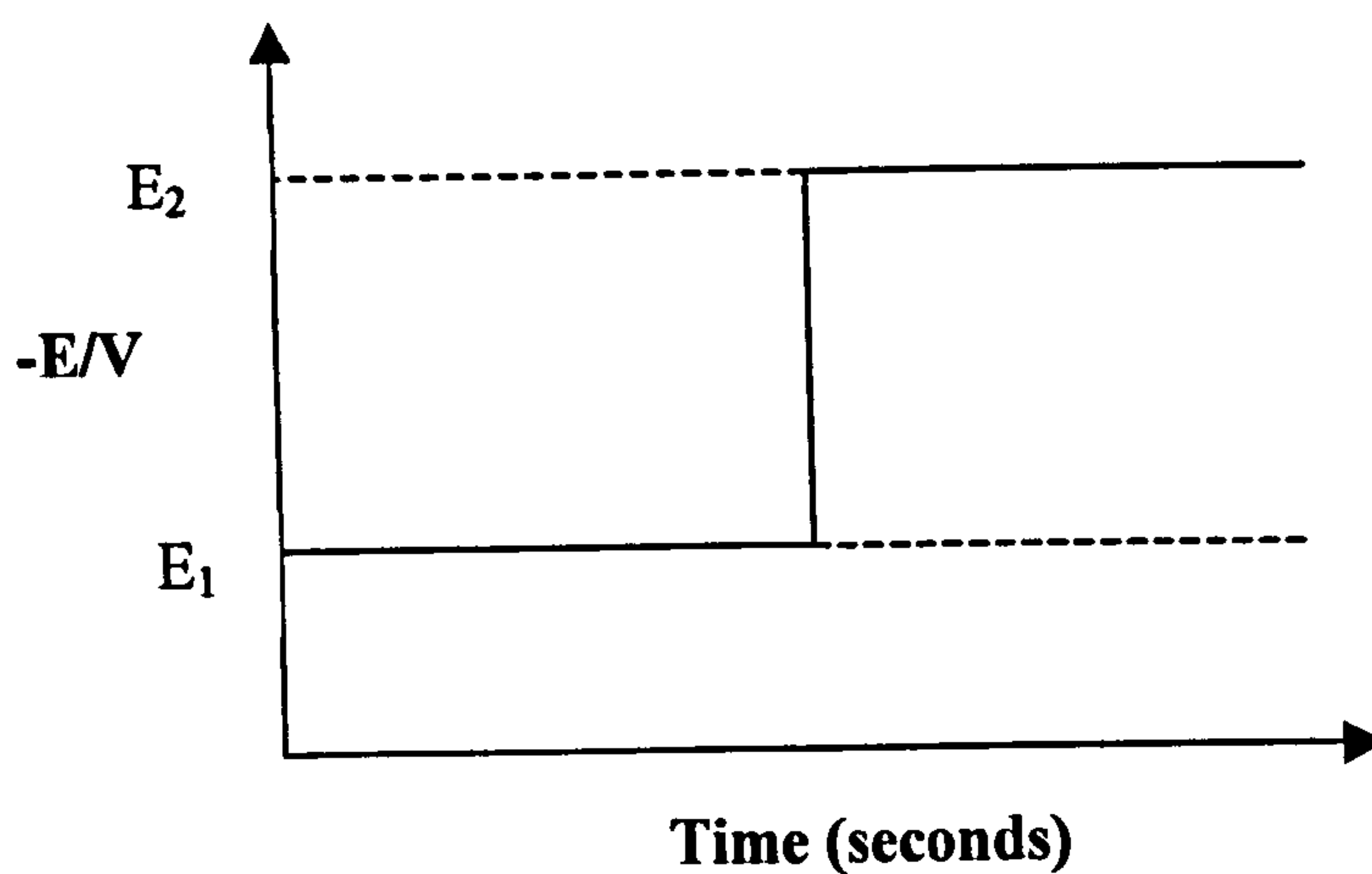
Potential Step Chronoamperometry was used for the polymerisation of homo- and co-polymer modified electrodes as previously mentioned. In Chapter 3 these polymers are compared to those developed using cyclic voltammetry, using X-ray Photoelectron Spectroscopy (XPS).

$$j = nFD^{1/2}c_R / \pi^{1/2}t^{1/2} \quad (\text{Equation 2.3})$$

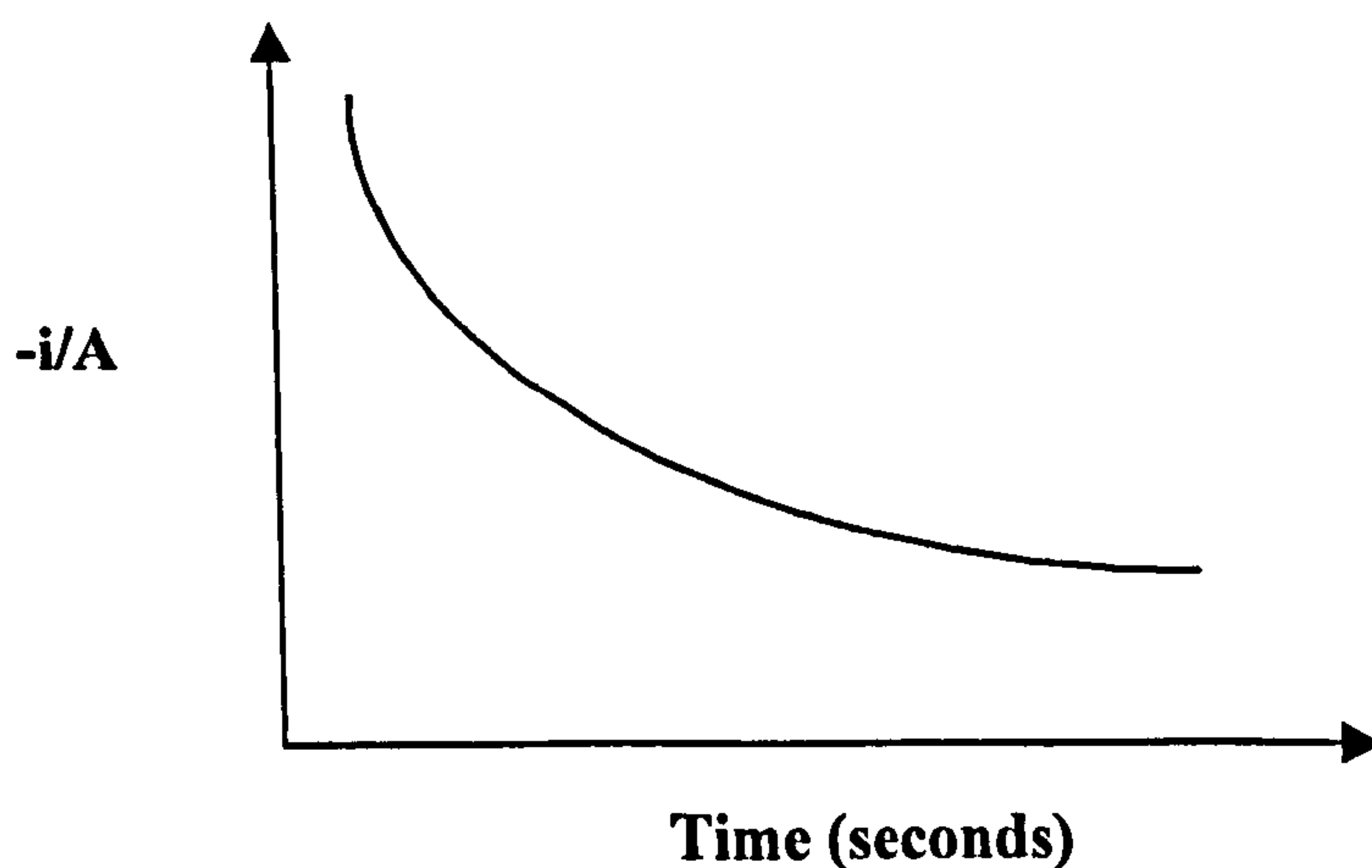
(where  $j$  is the experimental current density at potential  $E$ ,  $D$  is the diffusion coefficient,  $n$  is the number of electrons transferred,  $F$  is the Faraday constant and  $t$  is time from the commencement of the experiment)

#### 2.2.2.2 Polymerisation of Polymer Films

Homopolymer and copolymer films can also be electropolymerised using potential step techniques, although a slight variation on the standard electrochemical set up was required. In this case the  $1 \text{ cm}^2$  working electrodes were scanned (at a scan rate of  $20 \text{ mVs}^{-1}$ ) up to  $1.15\text{V}$  and held at this potential for 60 seconds in the appropriate solution. Deposition solutions with varying ratios of PFP and Py were also used in these experiments to control the polymer composition. After being held at  $1.15\text{V}$  a reduction step then followed. Figure 2.9 shows the typical deposition curve for polymer films that were produced using this method. The deposition solution contains only the reduced species,  $R$ . When the electrode potential is suddenly changed from its starting value the anodic process  $R \rightarrow O + ne^-$  occurs, where  $O$  is the oxidised species.



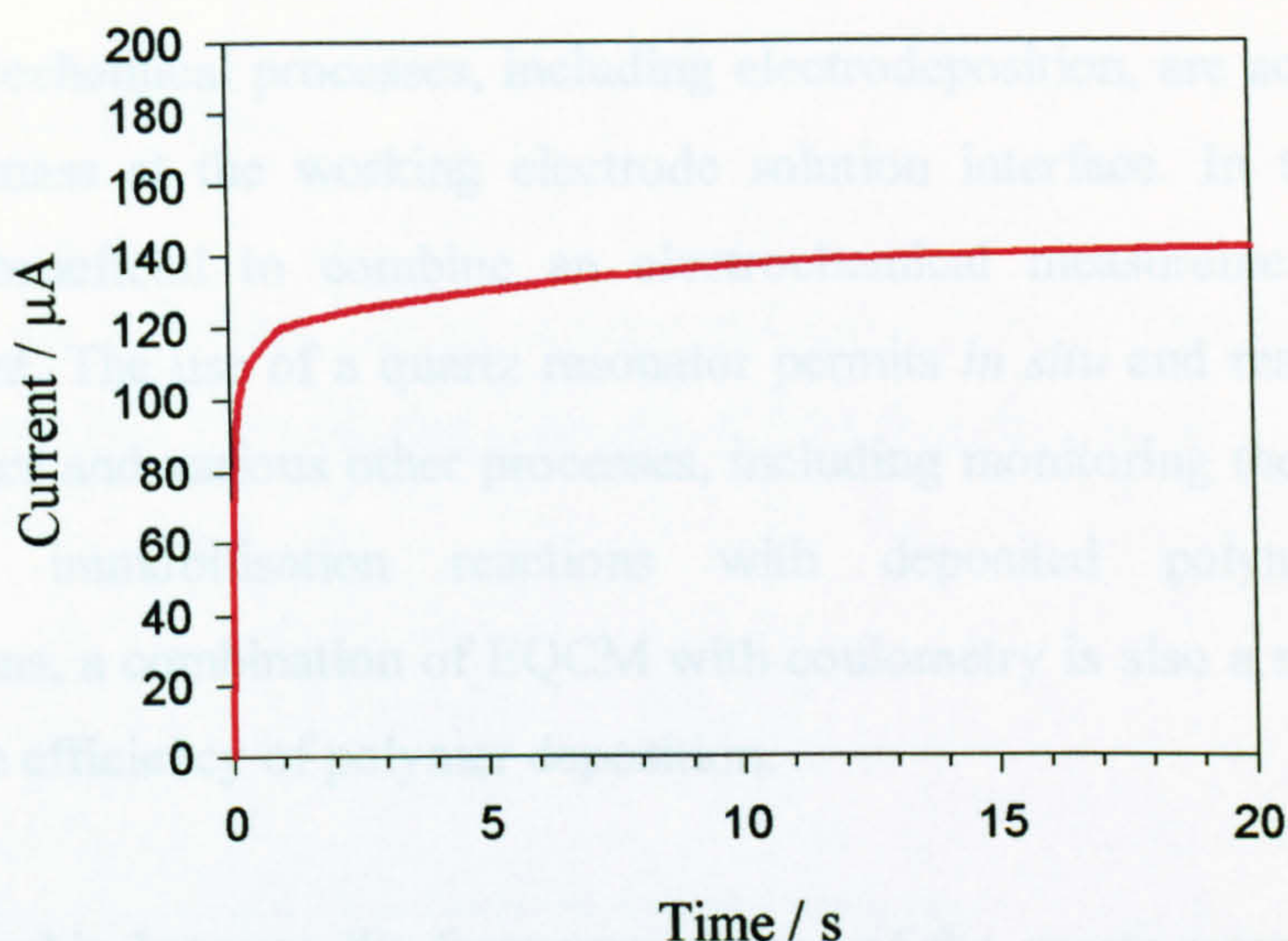
**Figure 2.7:** A graph illustrating the variation of the applied potential as a function of time in a potential step experiment. Using this method the potential is changed rapidly, usually from a current passes to one where the electrode reaction of interest takes place.



**Figure 2.8:** Graph illustrating the current response versus time in a potential step experiment. When the potential is increases this would initiate the very rapid oxidation of the monomer at the electrode surface. As the diffusion of the oxidised monomer decreases with time the current also decreases with time.



## 2.2.3.1 Background



**Figure 2.9:** A typical polymerisation deposition graph showing the relationship between polymerisation current and the time a step potential is applied. As the potential is increased there is a rapid increase in rate of diffusion of the oxidised monomer to the electrode surface, therefore, a rapid increase in current.

In Chapter 4, Section 4.2.1.1, a comparison is made between the reaction rates of polymers grown by this potential step method and that of those grown by cyclic voltammetry. Underlying this investigation was the observation that when the potential step method was used to produce polymer films, it was observed that less smooth films were produced compared to those formed during cyclic voltammetry. Thus it was hypothesised that altering the polymerisation method for the deposition of the polymers on the gold electrodes caused changes in the surface morphology and the internal microstructure of the films. In addition, in the case of copolymer films, the composition may be dependent on the deposition potential scheme used.



## 2.2.3 Electrochemical Quartz Crystal Microgravimetry<sup>9-11</sup>

### 2.2.3.1 Background

Many electrochemical processes, including electrodeposition, are accompanied by a change in mass at the working electrode solution interface. In these cases it is obviously beneficial to combine an electrochemical measurement with a mass measurement. The use of a quartz resonator permits *in situ* and real time probes of mass changes and various other processes, including monitoring the progress of any subsequent immobilisation reactions with deposited polymers. In these investigations, a combination of EQCM with coulometry is also a sensitive probe to estimate the efficiency of polymer deposition.

The relationship between the frequency change of the quartz resonator,  $\Delta f$ , and the mass change,  $\Delta m$ , is demonstrated by the Sauerbrey equation:

$$\Delta f = -[2f_0^2(A\sqrt{\mu_2\rho})^{-1}]\Delta m \quad (\text{Equation 2.4})$$

$\Delta f$  is the change in frequency,  $\Delta m$  is the change in mass,  $f_0$  is the fundamental crystal resonance frequency of the crystal in Hz (and was 10 MHz in this study),  $A$  is the electrode area,  $\mu$  is the sheer wave velocity and  $\rho$  is the density. Note the negative sign, which indicates that an increase of mass causes a decrease of the frequency from its original resonance condition. The frequency was measured as a difference between the working electrode crystal and a reference crystal.

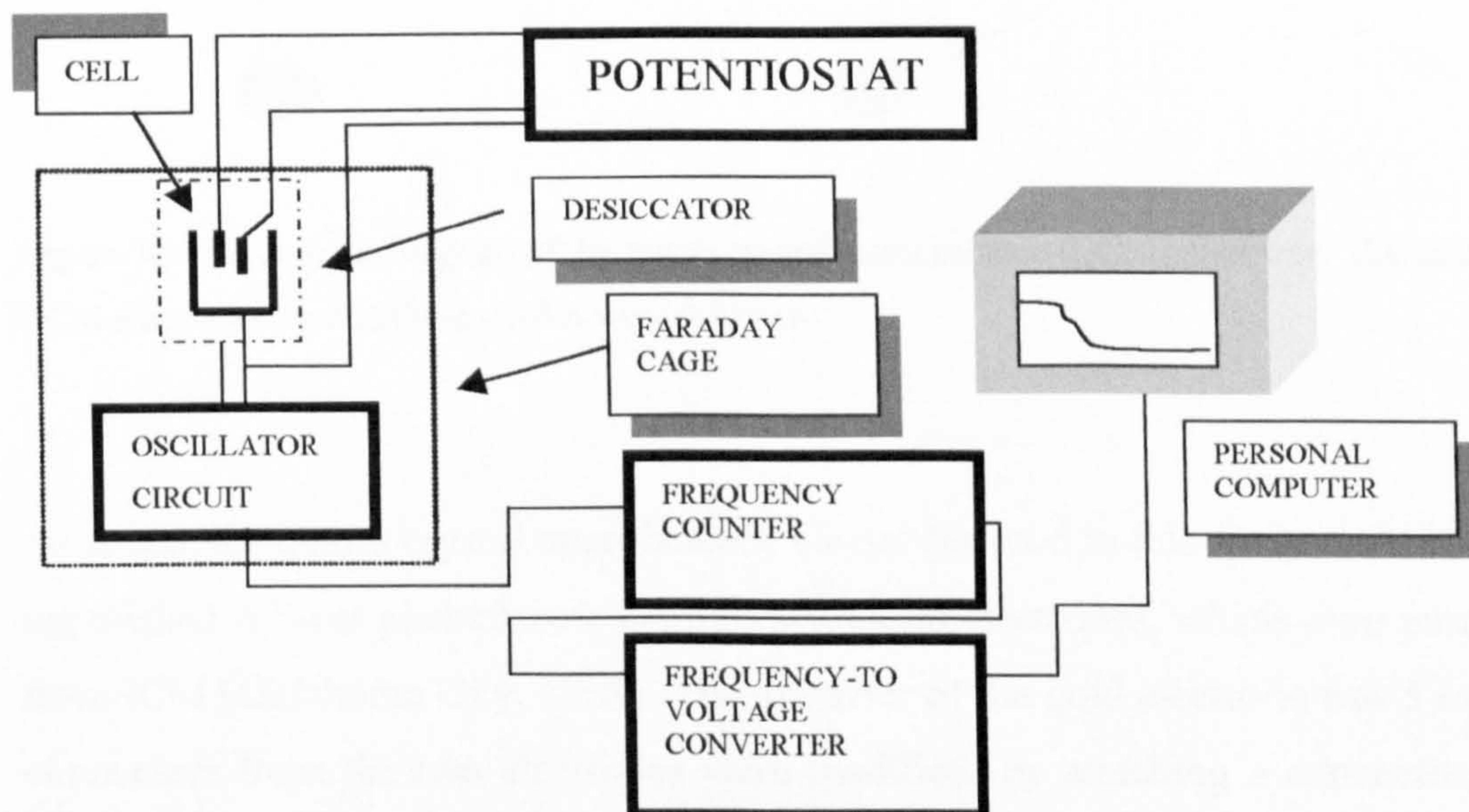
It has previously been found that although the Sauerbrey equation holds for rigid (or acoustically thin) films<sup>11</sup>. Deviations in the linear relationship between mass and frequency are found if these films are rubbery, fluid or rough (i.e. acoustically thick). This technique is ideal in monitoring the mass of dry material deposited on the quartz crystal for the investigations described below. However the following caveat should be considered, when quantitatively interpreting the measurements: problems may arise from changes in viscoelastic properties due to the fact that changes in polymer morphology are dependent on the polymer deposition process, solvent and film



history. To circumvent these difficulties, measurements were also carried out on dry films in an evacuated desiccator (note, the use of the desiccator removes any influence on the measurements due to sorbed water vapour).

### 2.2.3.2 Instrumentation

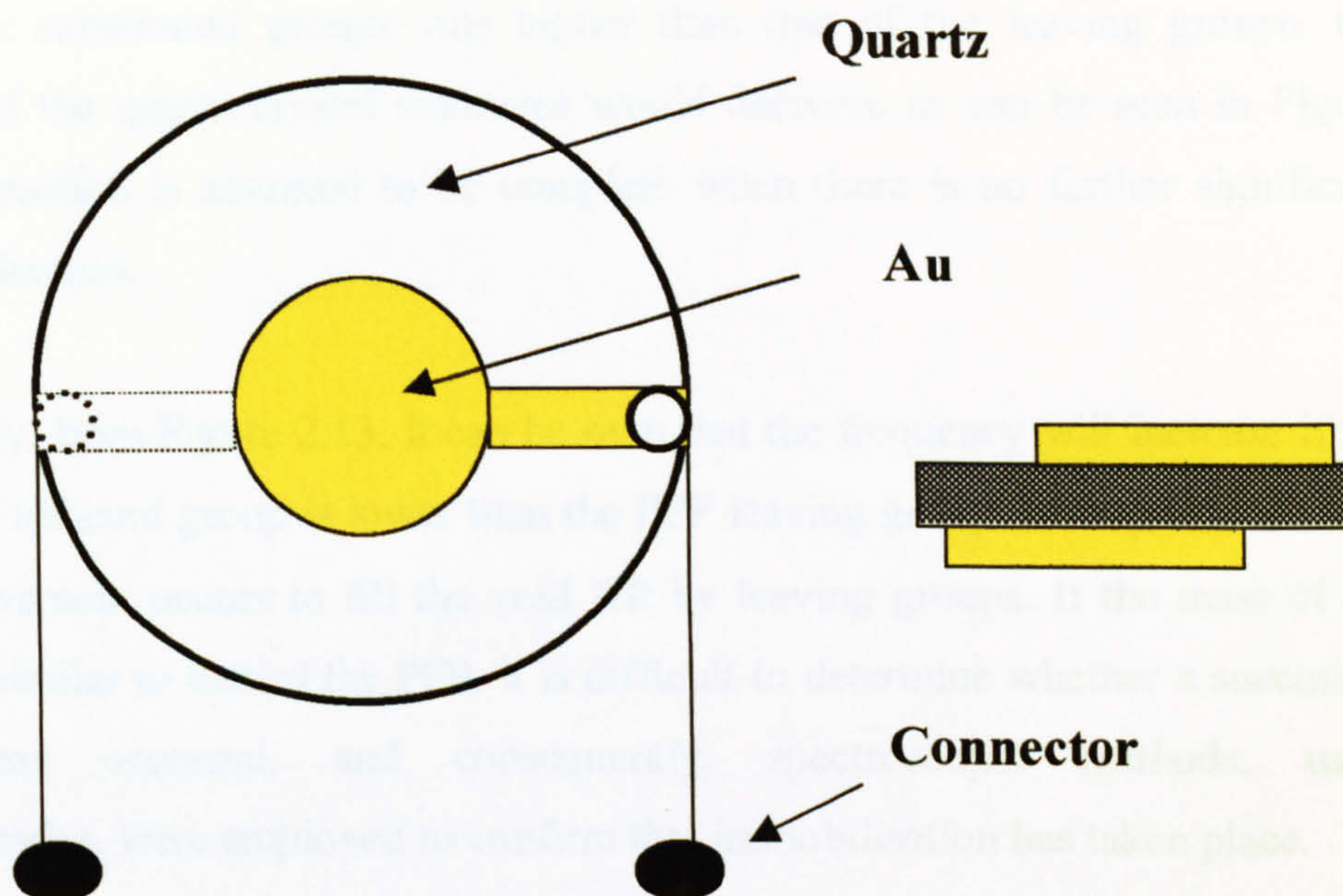
The typical set up of an EQCM is seen in Figure 2.10. A Faraday cage was also present to stabilise the cell and circuitry (protecting the circuitry from changes in temperature).



**Figure 2.10:** Schematic Diagram of EQCM set-up. The key components are the electrochemical cell and oscillator circuit within a faraday cage. The frequency counter and the frequency voltage converter are connected to a computer that records the data.

The cell, oscillator circuit board and the batteries to power it were all enclosed in a Faraday cage, earthed to the potentiostat. The oscillator circuit board comprises two oscillator circuits, one for the working crystal and one for a reference crystal. A typical quartz crystal microbalance electrode is shown in Figure 2.11 and the polymer is deposited onto the central disc on one side of the crystal.





**Figure 2.11:** Schematic diagram of the quartz crystal microbalance (QCM) electrode. The area of the QCM electrodes used in these studies were  $0.229 \text{ cm}^2$ .

As stated, the quartz crystal microbalance electrodes used in this study were 10 MHz, unpolished AT-cut piezoelectric crystals with gold electrodes, which were purchased from ICM (Oklahoma City, USA). The diameter of the gold electrode was 5 mm and connectors from the two electrodes were modified, by attaching a connector, to fit the measurement system. The crystals were mounted onto the base of the electrochemical cell using non-corrosive silicone rubber (RTV 3145, Dow Corning). Care was taken that no glue approached the circular mass sensitive portion of the electrode, as this has a significant effect on the crystal oscillation.

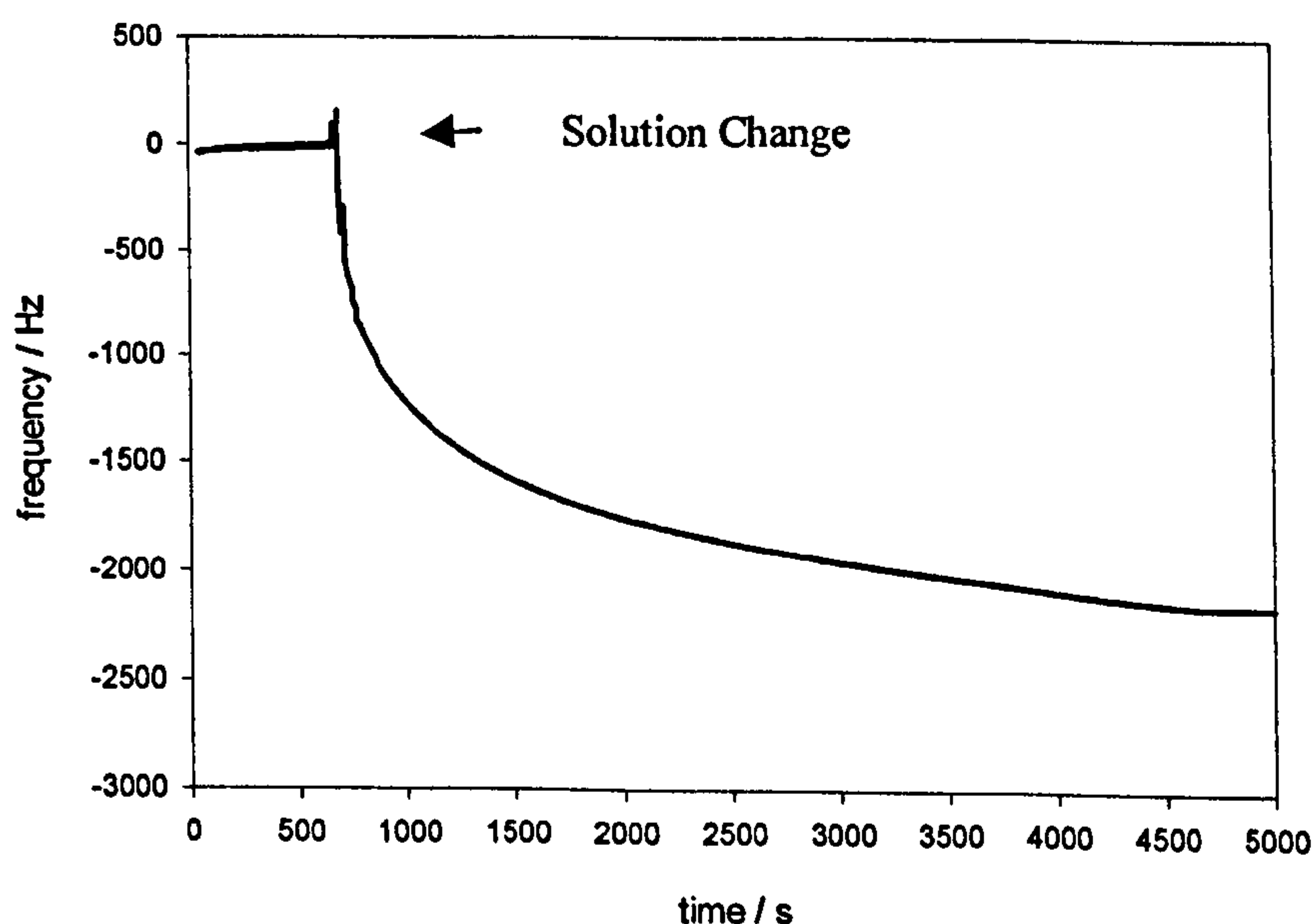
### 2.2.3.3 The Use of EQCM Gravimetry for Subsequent Immobilisation Reactions

EQCM gravimetry can be applied to functionalised, reactive polymers to follow subsequent *in situ* immobilisation reactions. The measured frequency change of the quartz resonator allows the calculation of the mass changes caused by the tethering

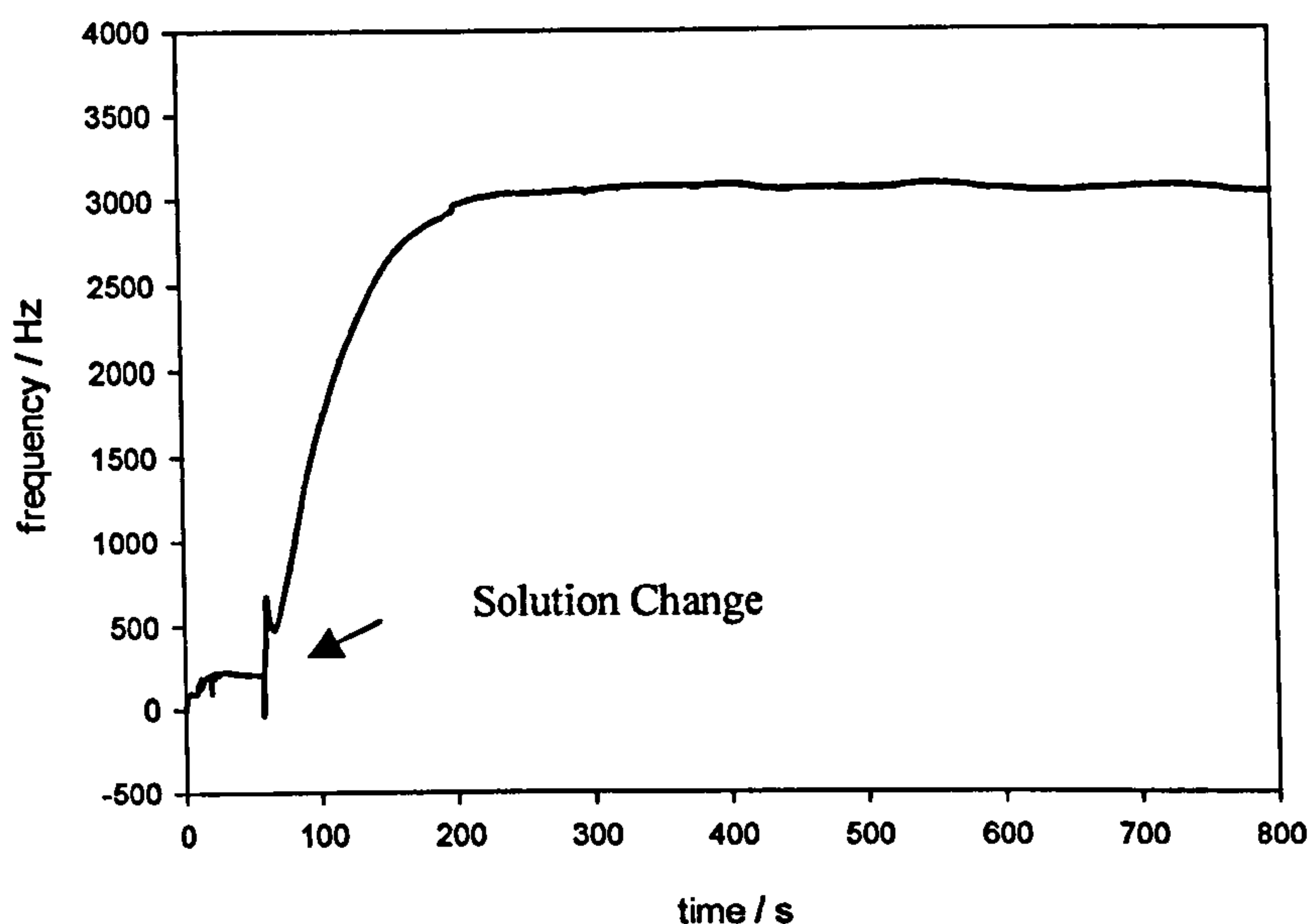


or immobilisation of the desired species. To allow successful measurement of the attachment of reactive species it is important that there is a significant difference in molecular weight compared to that of the PFP leaving group. For example, if the mass of the substituted groups was higher than that of the leaving groups, the frequency of the quartz crystal resonator would decrease as can be seen in Figure 2.12. The reaction is assumed to be complete when there is no further significant frequency changes.

Alternatively, from Figure 2.13, it can be seen that the frequency will increase if the mass of the tethered group is lower than the PFP leaving group. During this reaction solvent movement occurs to fill the void left by leaving groups. If the mass of the reactant is similar to that of the PFP, it is difficult to determine whether a successful reaction has occurred, and consequently, spectroscopic methods, using macroelectrodes, were employed to confirm that immobilisation has taken place.



**Figure 2.12:** Graph illustrating frequency decrease when homopolymer of PFP is reacted with a species (platinum containing solution) with a higher molecular weight than the pentafluorophenyl leaving group.



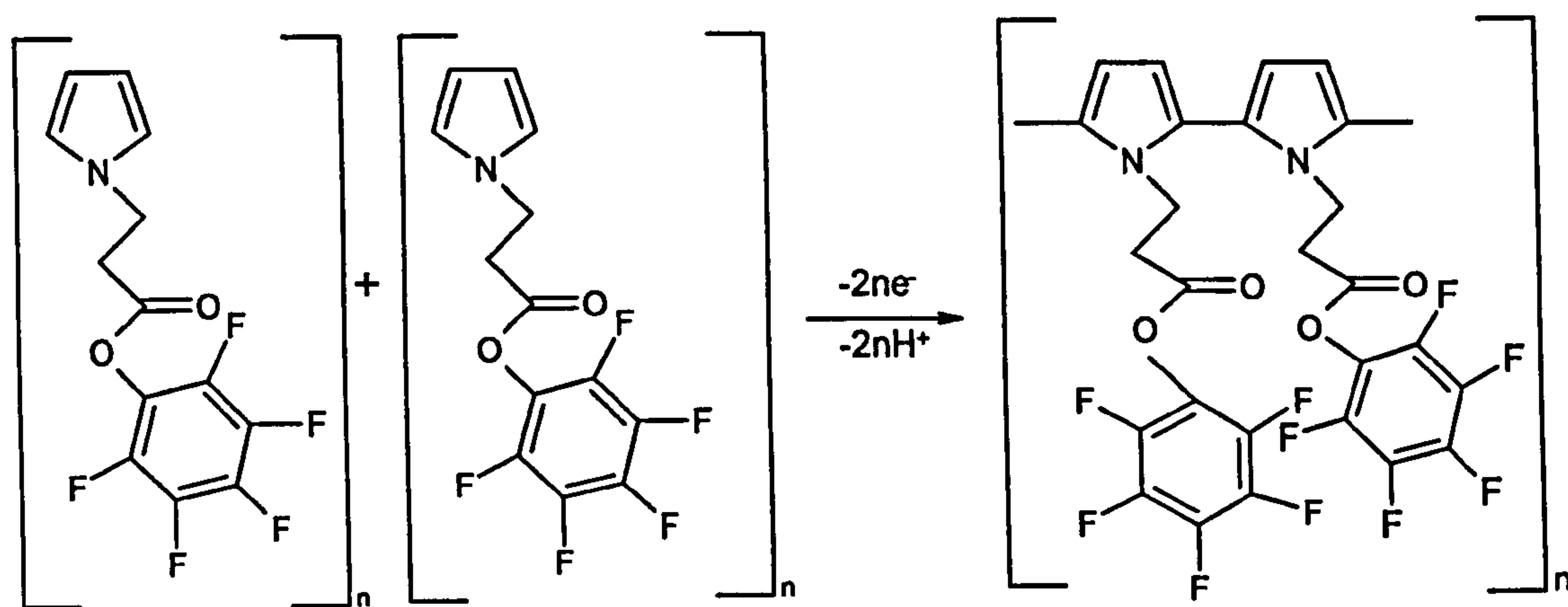
**Figure 2.13:** Graph illustrating frequency increase when homopolymer of PFP is reacted with a species (cystamine (see Chapter 5)) with a lower molecular weight than the pentafluorophenyl leaving group<sup>12</sup>.

## ***2.3 Illustrative Results from the Application of the Electrochemical Techniques***

### **2.3.1 Cyclic Voltammetry to Polymerise Poly(PFP)**

Typically, homopolymer films of PFP were electrodeposited (Figure 2.14) by immersing a 1 cm<sup>2</sup> gold electrode in an electrochemical cell containing a solution of 20mM PFP monomer and 0.1M tetraethylammonium perchlorate in acetonitrile. During the polymerisation process, using cyclic voltammetry, simultaneous doping (influx of perchlorate ions during oxidation) then undoping (efflux of perchlorate ions during reduction) and efflux of perchlorate ions of the polymer occurs

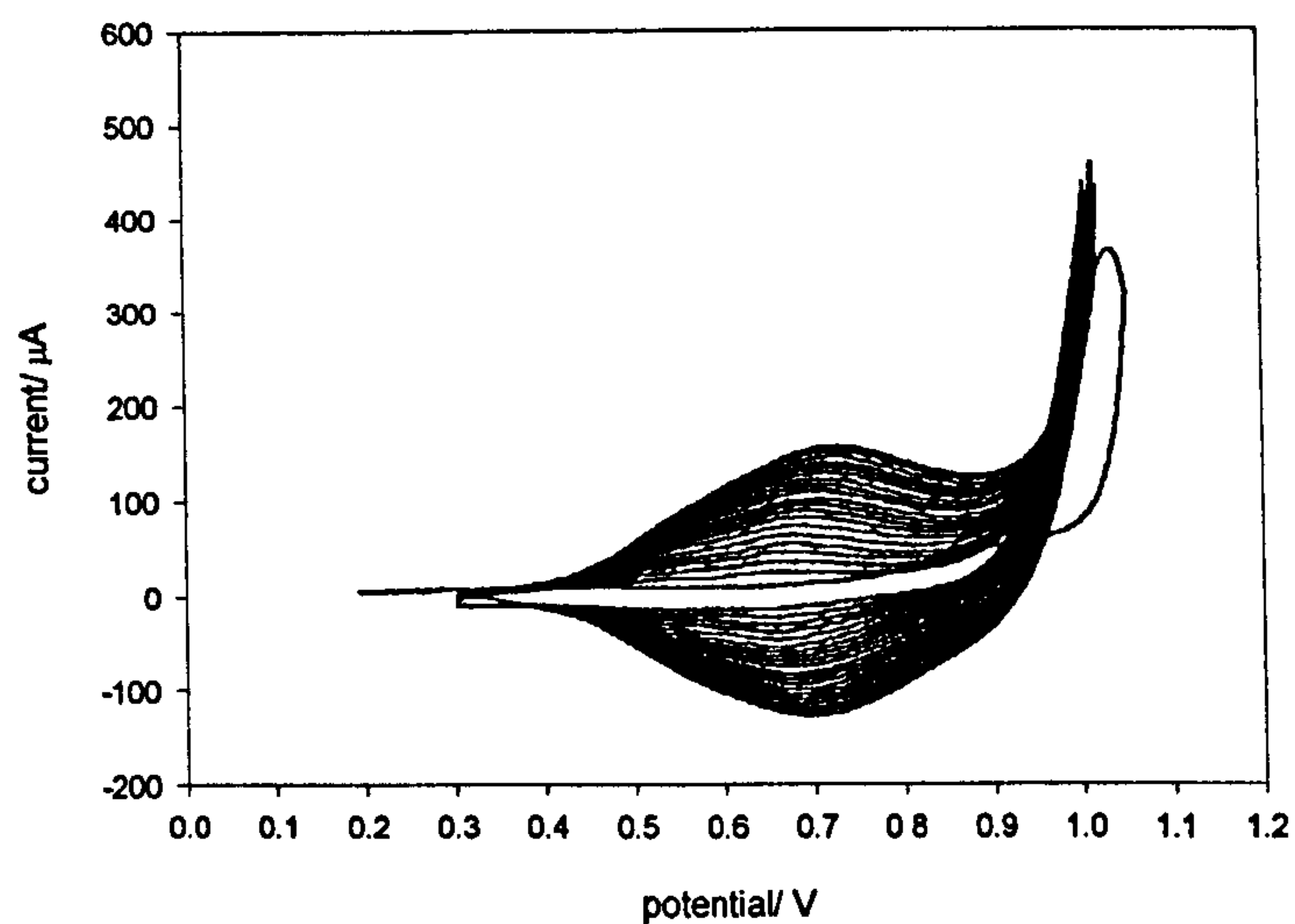
accompanied by the influx and efflux of perchlorate ions during the redox cycle. For polymer nucleation<sup>5</sup> to occur, the working electrode must be scanned to a maximum voltage of between 1.0 and 1.1 V. To obtain films of different thicknesses the electrode was then scanned to a specified potential limit, in the deposition solution, for different numbers of cycles.



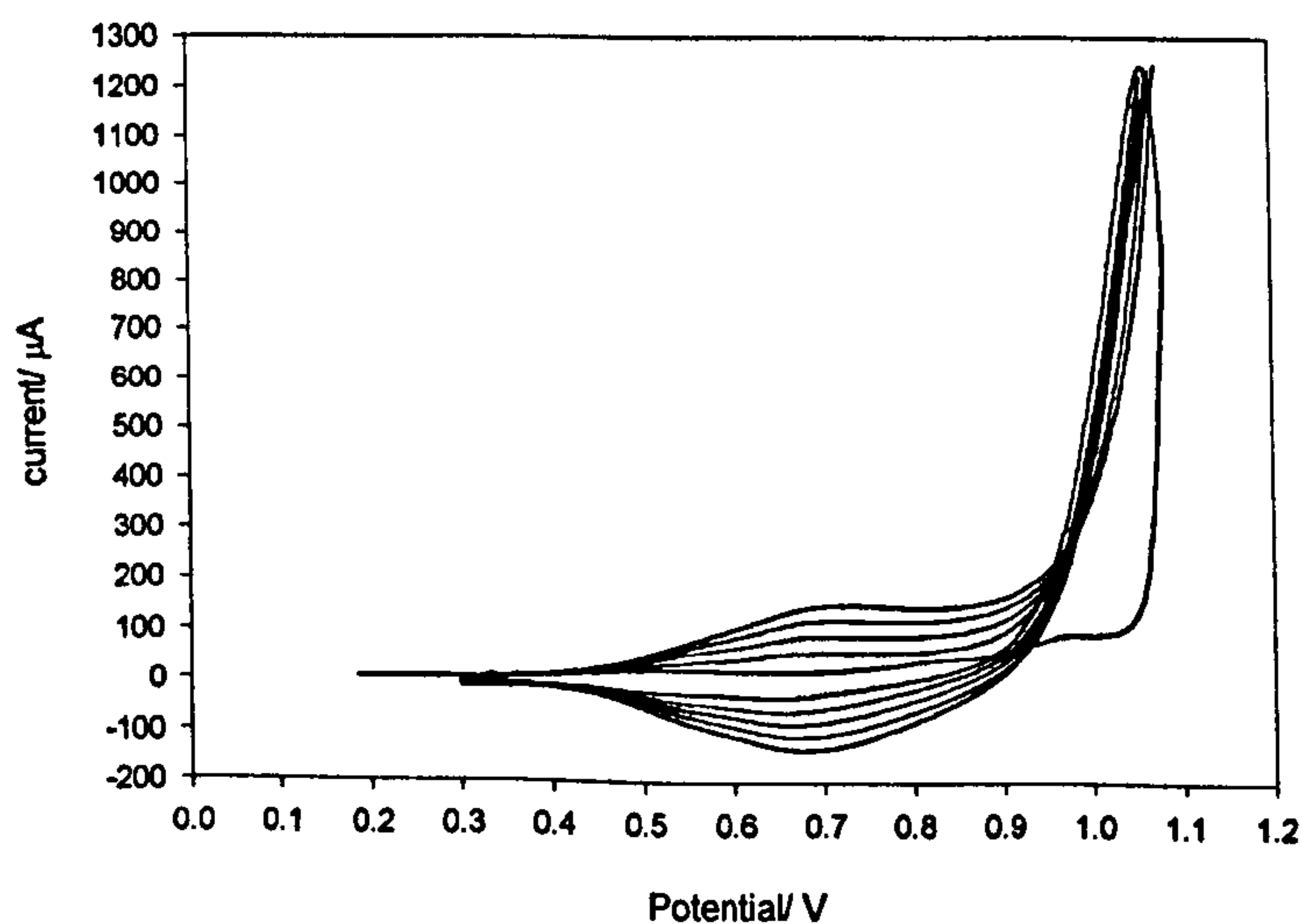
**Figure 2.14:** Reaction scheme illustrating the electrochemical polymerisation of a homopolymer of PFP. AS the potential increases the PFP monomer species oxidises and polymerises to form poly(PFP) on the electrode surface. The polymer is reduced as the potential decreases.

Attempts to control the polymers internal microstructure involved scanning the working electrode to two different potential limits, thereby, varying the rate of polymer deposition. For simplicity, these will be referred to as “slow” grown and “fast” grown poly(PFP) films. The slow grown poly(PFP) film was electropolymerised by potentially cycling the electrode from 0.2 V to 1.02V at a scan rate of 20 mVs<sup>-1</sup> (Figure 2.15).

The fast grown film was polymerised by scanning the potential to a slightly higher limit of approximately 1.05 V. Due to this slight increase in potential, the polymer reaches the desired anodic peak height (the same as the slow grown film), by cycling across the potential range fewer times. This can be seen in Figure 2.16 and this technique (of varying the upper potential limit) can also be used to control the rate of deposition of the homopolymer.



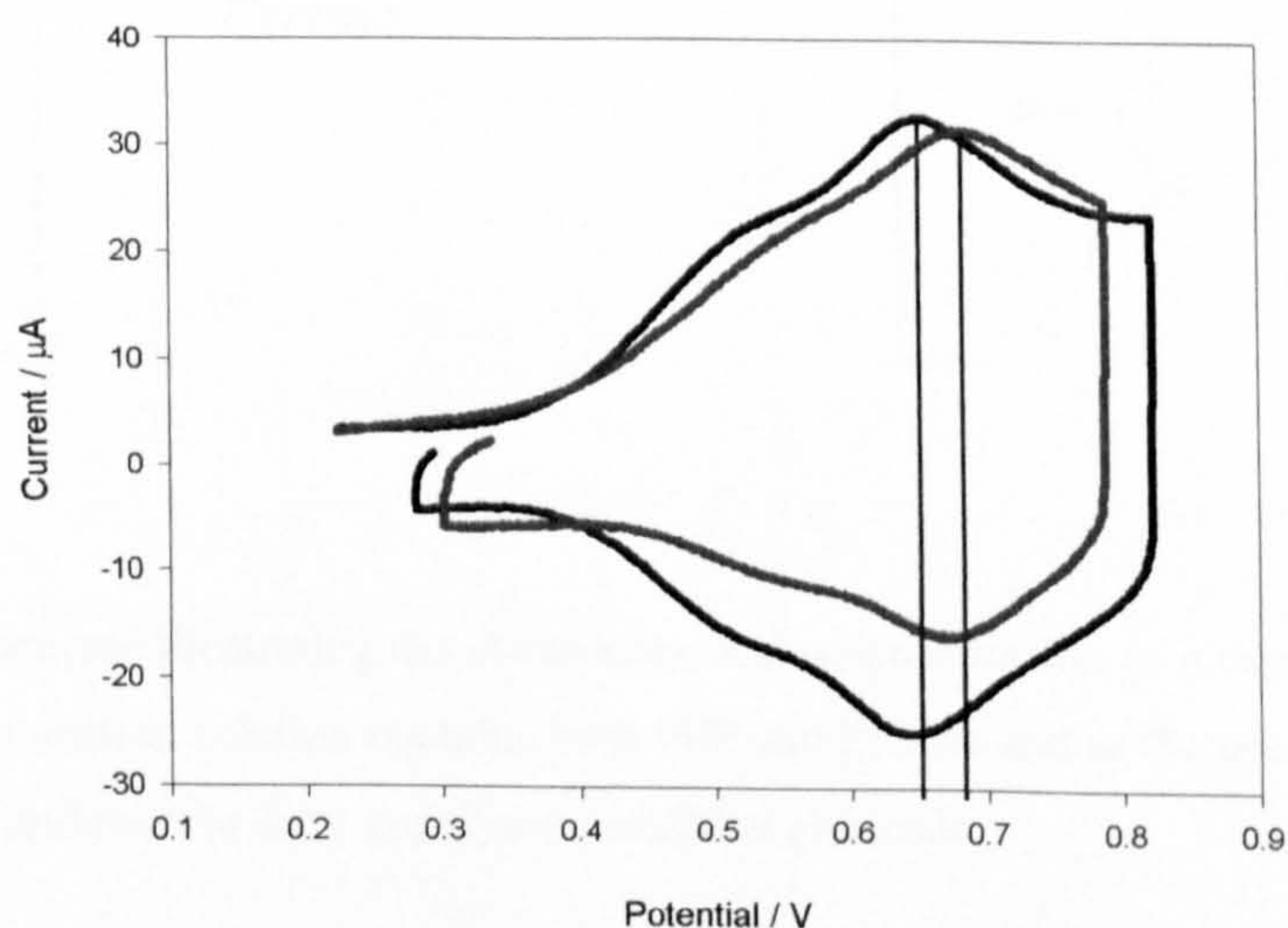
**Figure 2.15:** Cyclic voltammogram of the electrochemical polymerisation of the slow grown homopolymer of PFP by scanning the potential to 1.02 V in a deposition solution of 20 mM PFP monomer, 0.1 M TEAP and acetonitrile. Electrode area=1 cm<sup>2</sup> and reference electrode Ag|AgCl and scan rate of 20 mVs<sup>-1</sup>



**Figure 2.16:** Cyclic voltammogram of the electrochemical polymerisation of the fast grown homopolymer of PFP by scanning the potential to 1.05 V in a deposition solution of 20 mM PFP monomer, 0.1 M TEAP and acetonitrile. Electrode area=1 cm<sup>2</sup> and reference electrode Ag|AgCl and scan rate 20 mVs<sup>-1</sup>.



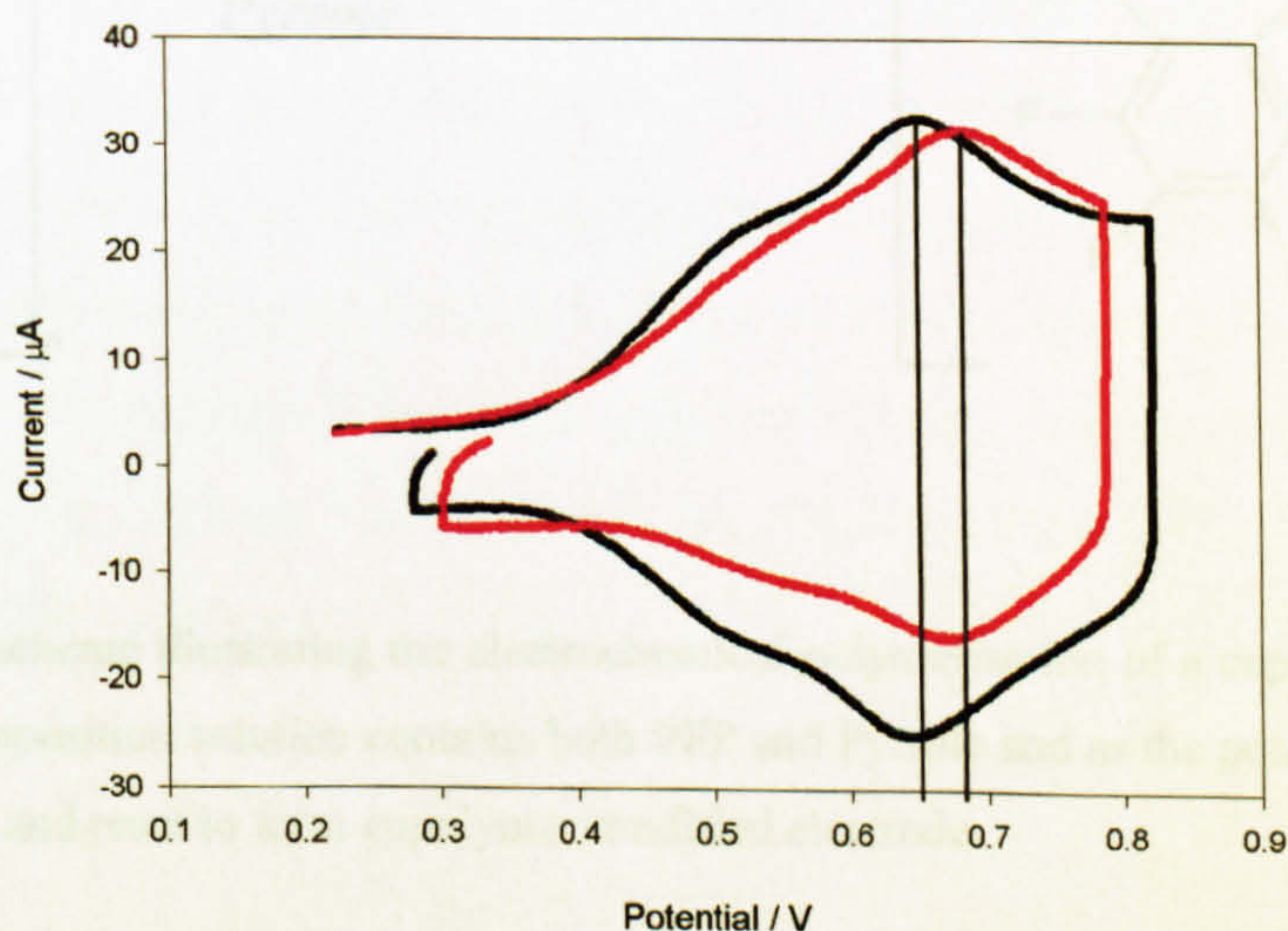
Cyclic voltammetry in background (monomer free) electrolyte was also used to indicate whether there might be any differences in microstructure between the fast and slow grown homopolymer, as a consequence of using different polymerisation parameters. This is illustrated by the observed change in the electrochemical oxidation potentials of both films when scanned in a background 0.1 M TEAP/MeCN solution (from 0 to 0.8 V). When the cyclic voltammograms of both films are compared, it can be observed (Figure 2.17) that there is a shift in oxidation peak potentials of approximately 0.03 V. This observable difference suggests that there was a change in the microstructure (the form and structure of the polymer beneath the surface of the film taking into account the space within the film that can be occupied by solvent or air) of both films as a consequence of increasing the potential limits during polymerisation. This difference could be accounted for either by differences of accessibility (ionic and/or electronic) of the “redox” sites or differences in the ease with which oxidation state induced conformational changes in the polymer can occur.



**Figure 2.17:** Cyclic voltammograms of the homopolymers of PFP after growth by scanning potential to 1.02V (red line) and by scanning potential to 1.05 V (black line). Polymer coated electrodes were 1 cm<sup>2</sup>, potential scanned from 0.2 V to 0.8 V in 0.1 M TEAP and acetonitrile solution vs. Ag|AgCl reference electrode at a scan rate of 20 mVs<sup>-1</sup>. Note the shift in oxidation peak of 0.03 V.



Cyclic voltammetry in background (monomer free) electrolyte was also used to indicate whether there might be any differences in microstructure between the fast and slow grown homopolymer, as a consequence of using different polymerisation parameters. This is illustrated by the observed change in the electrochemical oxidation potentials of both films when scanned in a background 0.1 M TEAP/MeCN solution (from 0 to 0.8 V). When the cyclic voltammograms of both films are compared, it can be observed (Figure 2.17) that there is a shift in oxidation peak potentials of approximately 0.03 V. This observable difference suggests that there was a change in the microstructure (the form and structure of the polymer beneath the surface of the film taking into account the space within the film that can be occupied by solvent or air) of both films as a consequence of increasing the potential limits during polymerisation. This difference could be accounted for either by differences of accessibility (ionic and/or electronic) of the “redox” sites or differences in the ease with which oxidation state induced conformational changes in the polymer can occur.

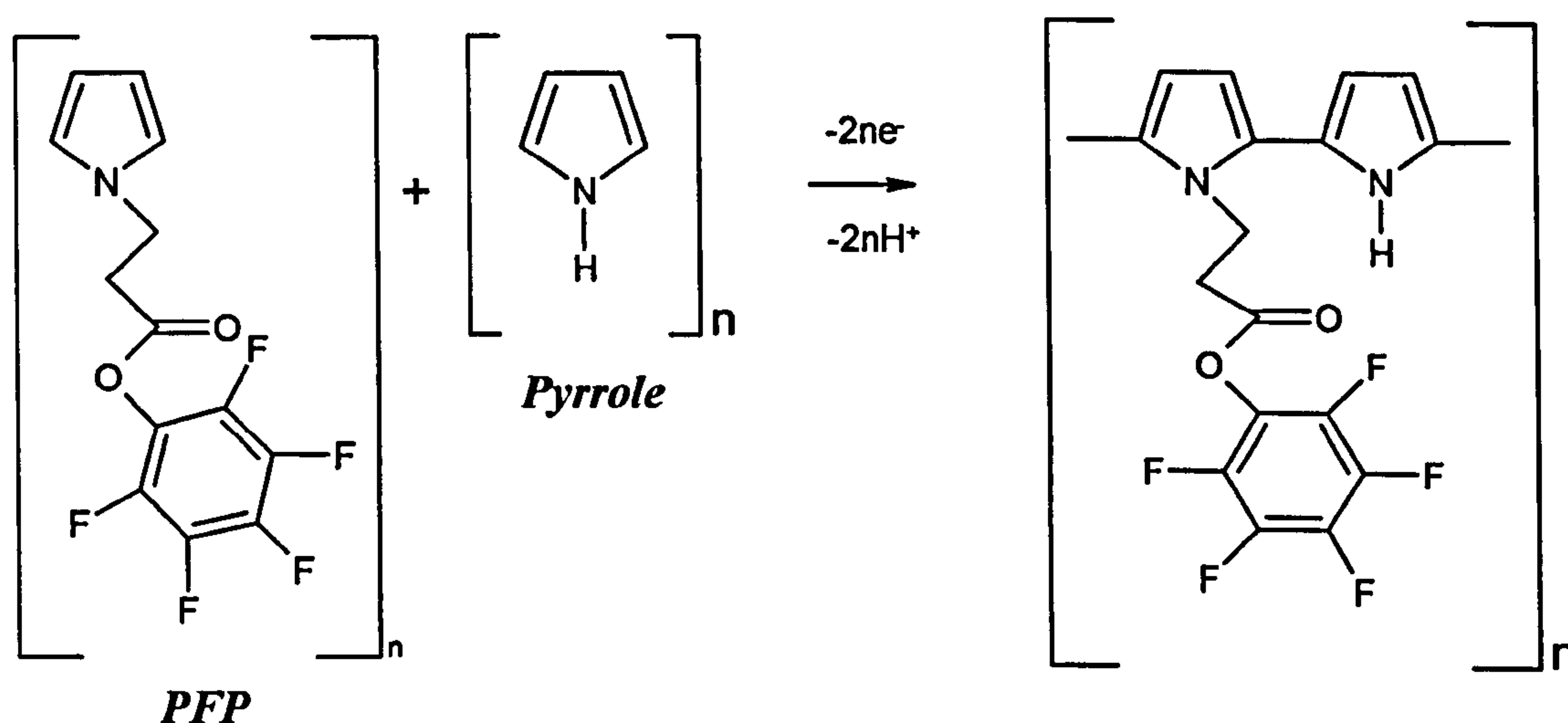


**Figure 2.17:** Cyclic voltammograms of the homopolymers of PFP after growth by scanning potential to 1.02V (red line) and by scanning potential to 1.05 V (black line). Polymer coated electrodes were 1 cm<sup>2</sup>, potential scanned from 0.2 V to 0.8 V in 0.1 M TEAP and acetonitrile solution vs. Ag|AgCl reference electrode at a scan rate of 20 mVs<sup>-1</sup>. Note the shift in oxidation peak of 0.03 V.



### 2.3.2 Use of CV to the Polymerise Poly(Py/PFP) Copolymer Films

Copolymer films were also electropolymerised using cyclic voltammetry (Figure 2.18). To produce the copolymer films of PFP and pyrrole, the same experimental set-up for homopolymer electrosynthesis was used and the deposition solution contained the desired molar ratios of PFP and Py monomers in dry acetonitrile and 0.1M TEAP solution. The scan rate was  $20 \text{ mVs}^{-1}$  versus Ag|AgCl reference. In general, copolymer films were prepared from deposition solutions containing ratios of 98:2, 95:5, 85:15, 75:25 or 50:50 PFP:Py monomer (these ratios were guided by the XPS compositional analysis, described in Chapter 3, section 3.4.2.1).



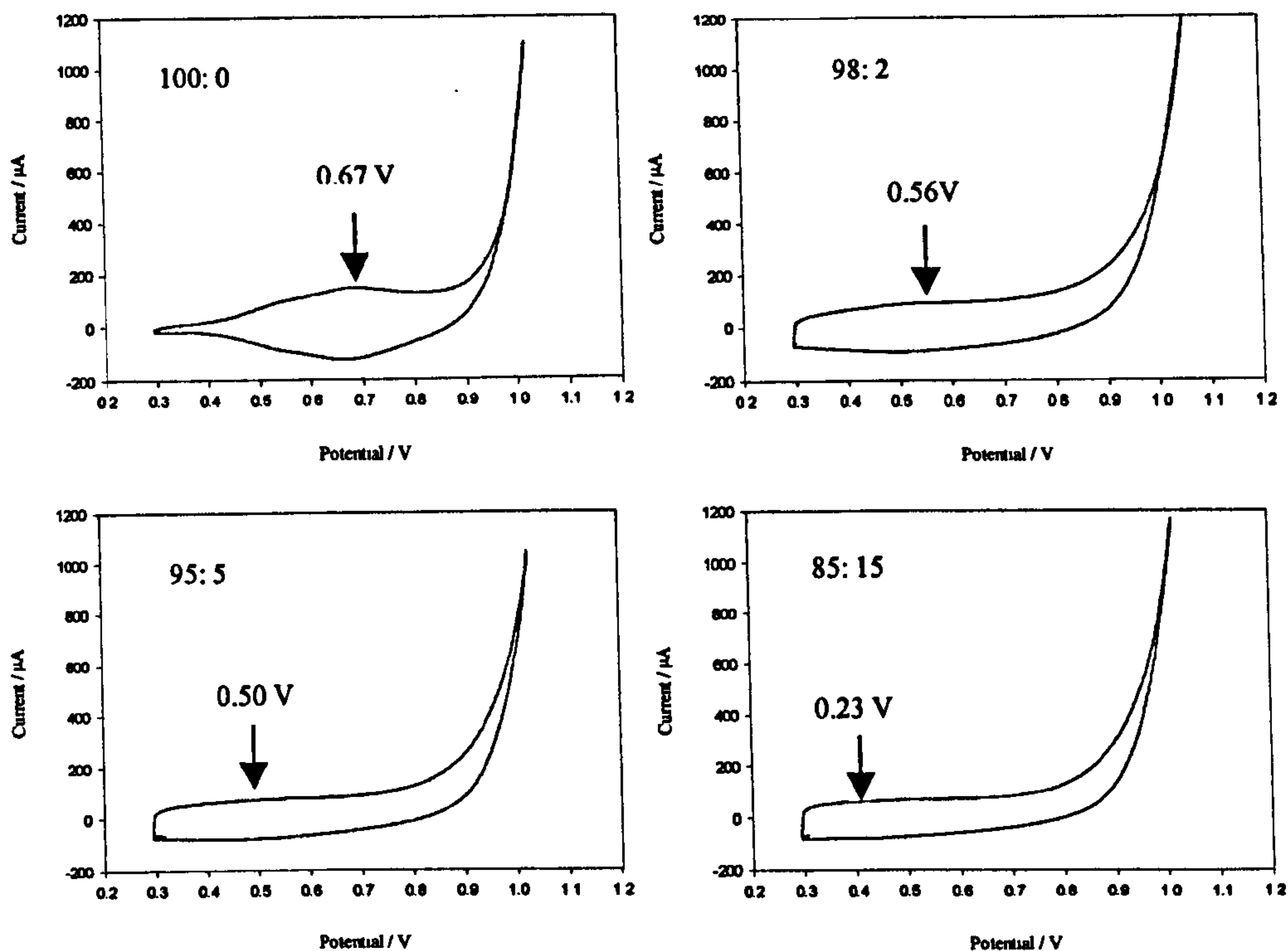
**Figure 2.18:** Reaction scheme illustrating the electrochemical polymerisation of a copolymer film of PFP and Pyrrole. The deposition solution contains both PFP and Pyrrole and as the potential increases both monomers oxidise and react to form copolymer modified electrode.

The deposition solution was prepared by adding a small volume of 1M pyrrole and acetonitrile solution to the electrolyte-containing PFP solution.  $1 \text{ cm}^2$  gold working electrode was immersed in the solutions and scanned to a potential of approximately 1.0 V. As the mole fraction of pyrrole increases, the potential required for polymer nucleation and growth to occur reduces. It was thus relatively easy to produce copolymers of PFP with pyrrole, although, due to the observation that a deposition solution containing ca. 5% pyrrole monomer was required to give a copolymer with



50% pyrrole, care was taken to accurately prepare the copolymer solutions. The specific ratios of monomer units incorporated in the polymer film are discussed in Chapter 3, Section 3.4.2.1. The difference between the deposition solution and resultant copolymer composition occurs due to the difference in monomer oxidation potential of pyrrole and PFP. Comparison of oxidation potential from cyclic voltammetry shows that PFP has a higher value of approximately 1.1 V compared to Py, which has a value of 0.8 V. This change in monomer oxidation potential is due to the substitution at the N position of the pyrrole ring. It has previously been established that N-alkyl substitution (for short chain alkyl groups) is expected to cause little effect on the potential of monomer oxidation<sup>14</sup>. However, when the substituents are larger, they can both influence the redox potential of the monomer, as well as cause steric hindrance during the coupling of other monomer units. This leads to a non-linear relationship between deposition solution monomer compositions and that found in the resultant copolymer.

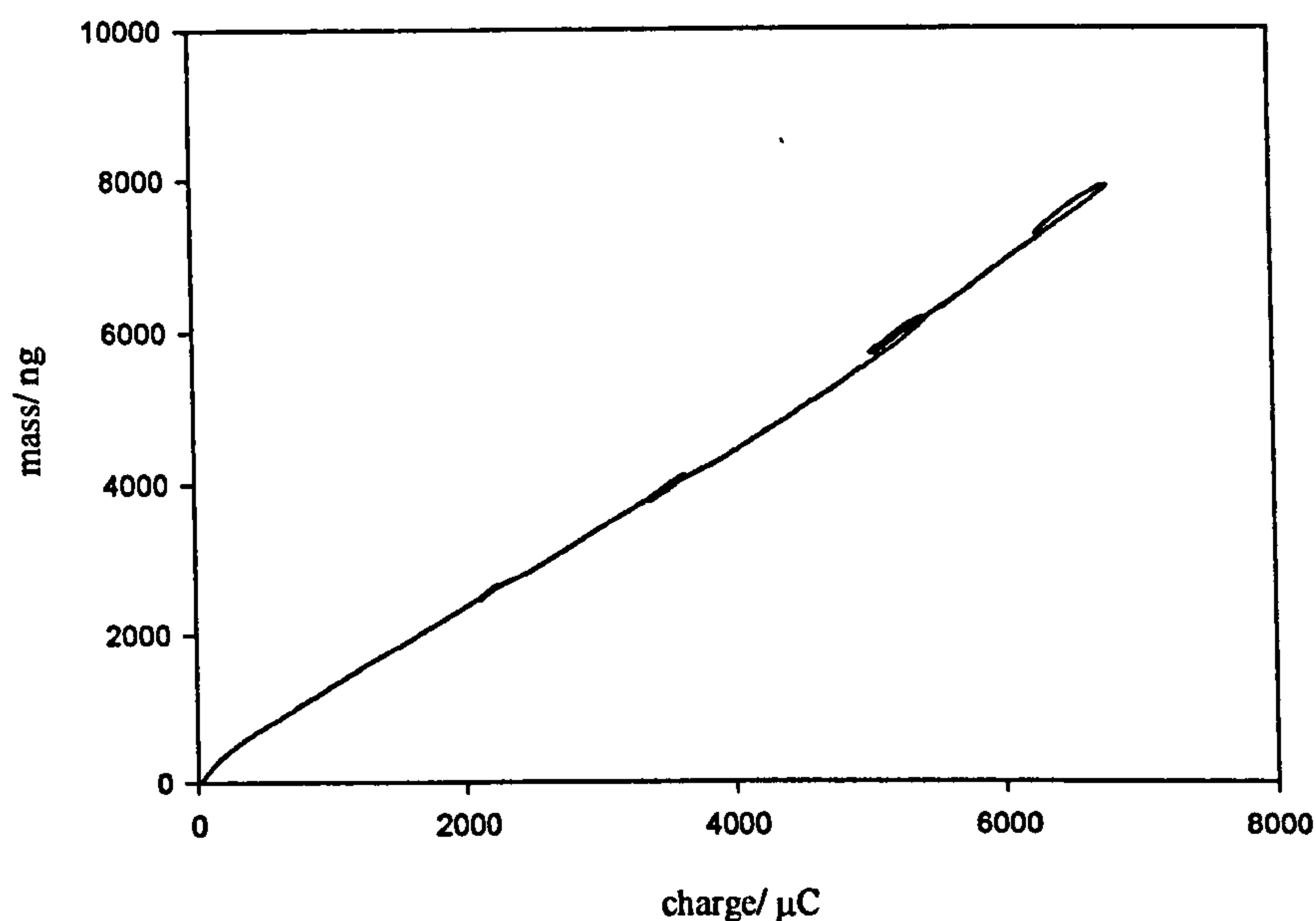
The cyclic voltammograms of the polymerisation of the copolymers can be seen in Figure 2.19. There is an observed shift in oxidation potential (reaching the same oxidation value of pyrrole) as the pyrrole content of the deposition solution increases. This indicates that there is an increase of pyrrole units incorporated within the polymer films as the molar concentration of pyrrole monomer increases in the deposition solution. The homopolymer and copolymer films described above can be modified further using various nucleophilic substitution reactions. This will be discussed further in the Chapters 4, 5 and 6.



**Figure 2.19:** Cyclic Voltammograms of the polymerisation of homopolymer of poly(PFP) from a deposition solution of 20 mM PFP, 0.1 M TEAP/acetonitrile (100: 0) and copolymers from a deposition solution containing 98: 2, 95: 5 and 85: 15 ratio of PFP: Py in 0.1 M TEAP and acetonitrile. Electrode area 1 cm<sup>2</sup>, Ag|AgCl reference electrode at 20 mVs<sup>-1</sup>. Note the decrease in oxidation potential (as indicated on the graph) as the amount of pyrrole incorporated in the film increases.

### 2.3.3 EQCM Measurements During the Polymerisation of Poly(PFP)

As indicated earlier, electrochemical polymerisation of the PFP monomer units by electrochemical methods is successful in forming polymer modified electrodes. During the polymerisation process, simultaneous doping then undoping of perchlorate ions occurs. This can be followed using EQCM measurements and plotting the frequency (mass) changes against the electrochemical charge passed as in Figure 2.20.



**Figure 2.20:** QCM frequency changes (mass increase) for the polymerisation of poly(PFP). Quartz crystal working electrode was scanned in 20 mM PFP, 0.1 M TEAP and acetonitrile vs. Ag|AgCl reference electrode. Electrode area=0.229 cm<sup>2</sup> and scan rate of 20 mVs<sup>-1</sup>.

The efficiency of the polymerisation process can be calculated, using a value for the Faraday constant of 96,500 Cmol<sup>-1</sup> and assuming 2 moles of electrons are involved per monomer (1 mole) addition (see explanation for the assumption in Figure 2.21). By dividing the estimated gradient from the graph (Figure 2.20) by the charge needed for a 100% efficient process, the efficiency for the deposition condition used can be calculated. The efficiency of polymerisation in this example was approximately 50% (the absolute value of this will be dependant upon the counter ion used, the deposition potential and the purity of the monomer, particularly with respect to the presence of oligomers).

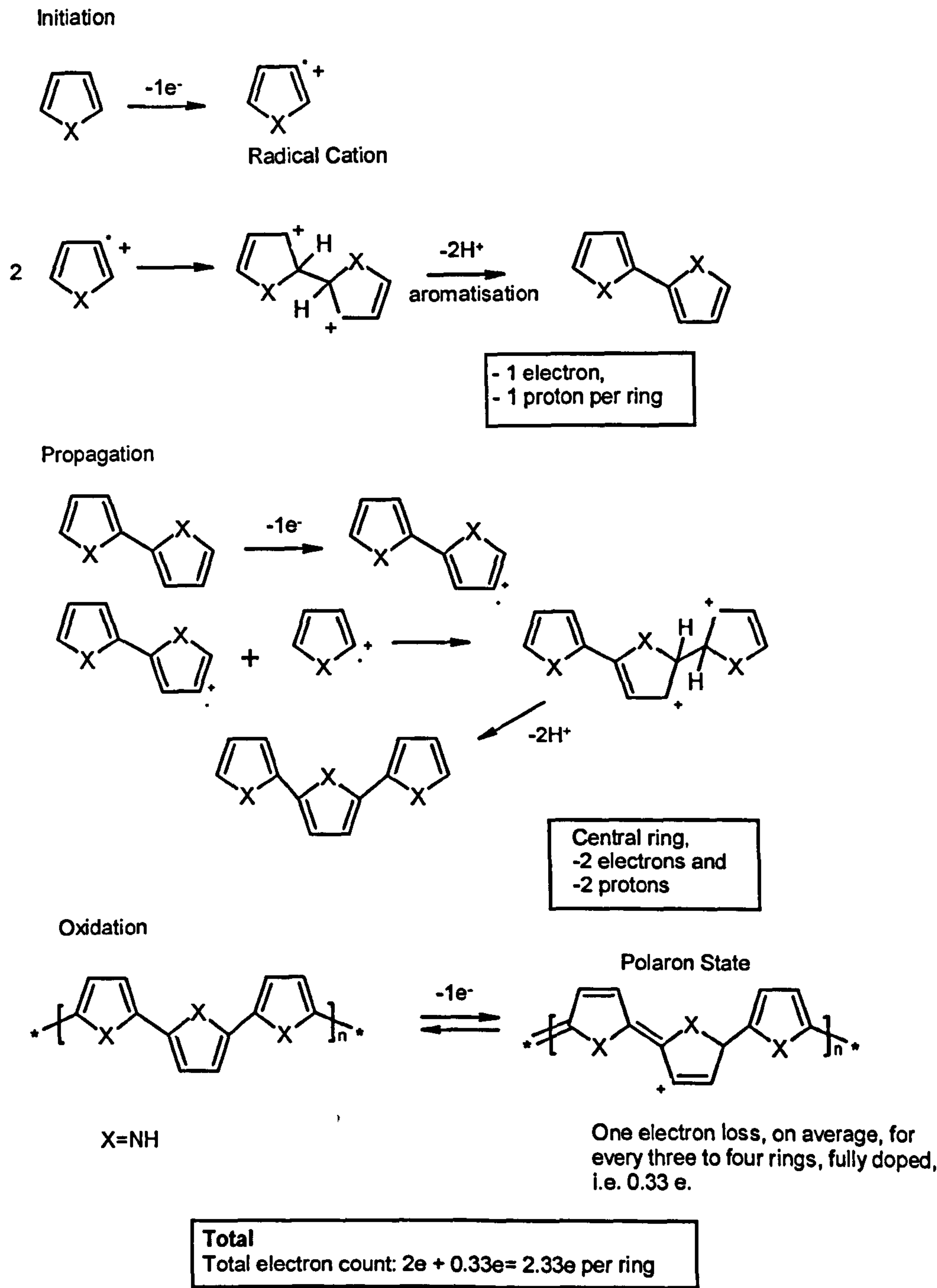
During the oxidation cycle, the polymeric film is doped before additional oxidised monomer adheres to the deposited polymer. It can be noticed that after the first nucleation cycle, the mass of the polymer film increases constantly during the



oxidation cycle. At low potential this increase in mass is a consequence of the influx of ions and solvation changes associated with the oxidative doping of the conducting polymer film. At higher potentials, the increase in mass corresponds to the deposition of new polymer material and further ion movement. To determine at what stage in the process that deposition of the polymer onto the electrode and the oxidative doping of this polymer occurs, EQCM measurements were obtained for the doping and undoping of the polymer film in, monomer free, electrolyte containing solution. These measurements show that the increase in mass of the polymer film in deposition solution and those in background electrolyte diverges at approximately 0.96 V (Figure 2.22 A). Above this potential, new polymer material starts to adhere to the polymer-modified electrode. Furthermore, the polymer mass continues to increase for the first 50 mV of the reduction cycle. The mass changes that occur at low potentials are due to the influx of ions and solvation changes associated with the oxidation of the polymer. At higher potentials the increase in mass is a consequence of the deposition of new polymer, and of continued ion and solvent movement. The potential required for monomer oxidation in a polymer surface is approximately 0.87V, although, polymer material does not adhere to the film until 0.96 V. This difference in potential is due to the need for the oxidised monomers to produce oligomers that adhere to the polymer surface. It was also established that during the first deposition cycle, where the polymer is initially deposited on the gold electrode, the potential at which monomer oxidation occurs is significantly higher than that required when oxidation takes place on a pre-existing polymer surface.

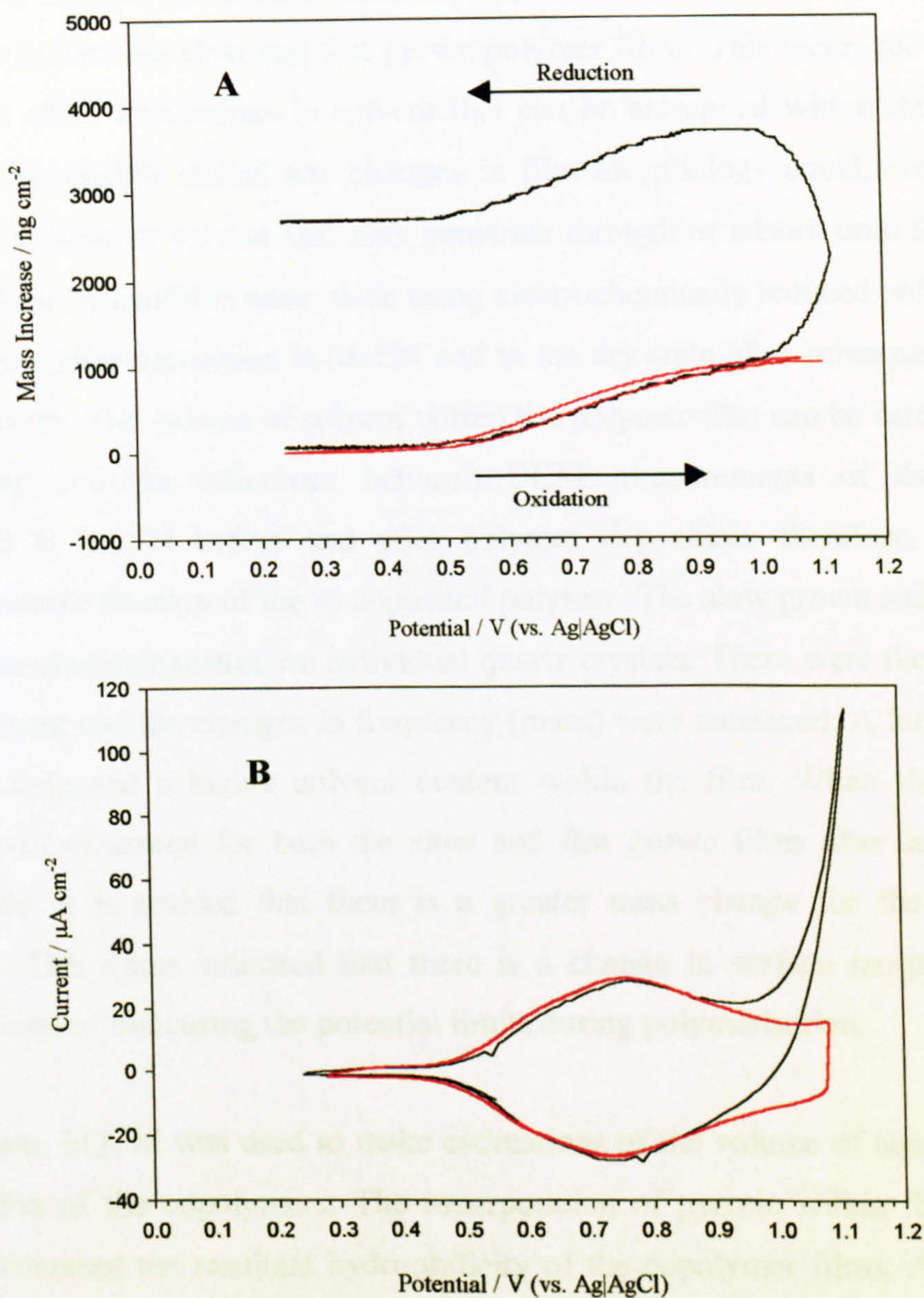
In addition, EQCM measurements were used to assay the amount of polymeric material deposited on the electrode. By changing the upper potential limit during the polymerisation process there is a direct affect on the deposition rate. It is possible to assess the rate of deposition of the polymer on the working electrode by the change in the mass of material that has been deposited on the working electrode. From Figures 2.23 and 2.24 it can be seen that when the potential limit is increased, the rate of deposition of polymer is also increased and consequently there is a more rapid decrease in frequency (as mass increases).

Using the method outlined previously, the coulometric efficiency of the polymerisation process for the fast and slow grown films is calculated as 77% and 50% respectively.



**Figure 2.21:** Mechanism showing the oxidative polymerisation of pyrrole. The same mechanism is followed for the polymerisation of PFP. Note that approximately 2.33 electrons are needed for the addition of each ring.





**Figure 2.22:** The electrochemical current (B) and the increase in polymer mass (A) during both the deposition (black line) of poly(PFP) and the subsequent redox cycling in background electrolyte: 0.1 M TEAP in MeCN (red line). Scan rate 20 mV/s, deposition solution 20 mM PFP, 0.1 M TEAP in MeCN and an electrode area 0.229 cm<sup>2</sup>.



### **2.3.4 EQCM Measurements of Solvated Polymer Films**

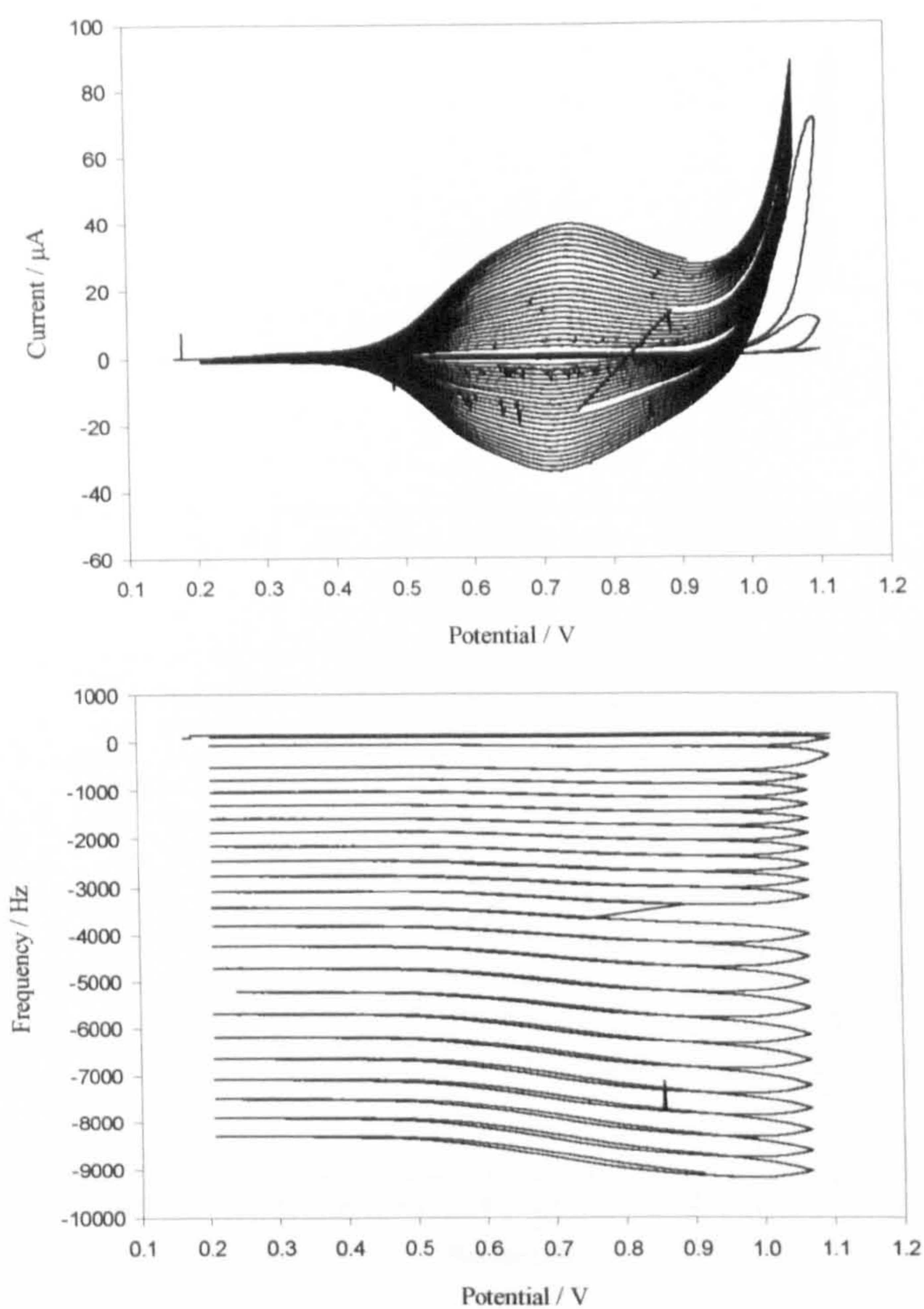
In addition, EQCM was used to establish whether there was any internal structural difference in both the slow and fast grown polymer films. This technique provides a method to allow estimations in solvent that can be entrapped within the deposited films. As previously stated, any changes in film morphology could, consequently, affect the volume of solvent that may penetrate through or adsorb onto the polymer matrix. These estimations were made using electrochemically reduced polymers both immediately after deposition in MeCN and in the dry state after subsequent removal of the solvent. The volume of solvent within the polymer film can be estimated from its density and the difference between QCM measurements of the electrode immersed in MeCN before and after polymer deposition, therefore, giving the solvent volume fraction of the as deposited polymer. The slow grown and fast grown films were electrodeposited on individual quartz crystals. These were then immersed in the solvent and the changes in frequency (mass) were measured. A larger increase in mass indicated a higher solvent content within the film. When the frequency change was measured for both the slow and fast grown films after immersion in acetonitrile it is noticed that there is a greater mass change for the fast grown polymer. This again indicated that there is a change in surface morphology as a consequence of increasing the potential limits during polymerisation.

In addition, EQCM was used to make estimations of the volume of aqueous solvent penetration of the copolymers. The incorporation of pyrrole within the polymeric matrix increased the resultant hydrophilicity of the copolymer films. A decrease in the number of hydrophilic PFP units within the film lead to the possibility of an increase in aqueous (water) penetration. To illustrate this copolymer films were deposited on individual quartz crystals. These were then immersed in solvent and the frequency changes of the films were recorded. These results show that as the amount of pyrrole incorporated in the copolymer film increased the amount of solvent penetration also increases<sup>15</sup>.



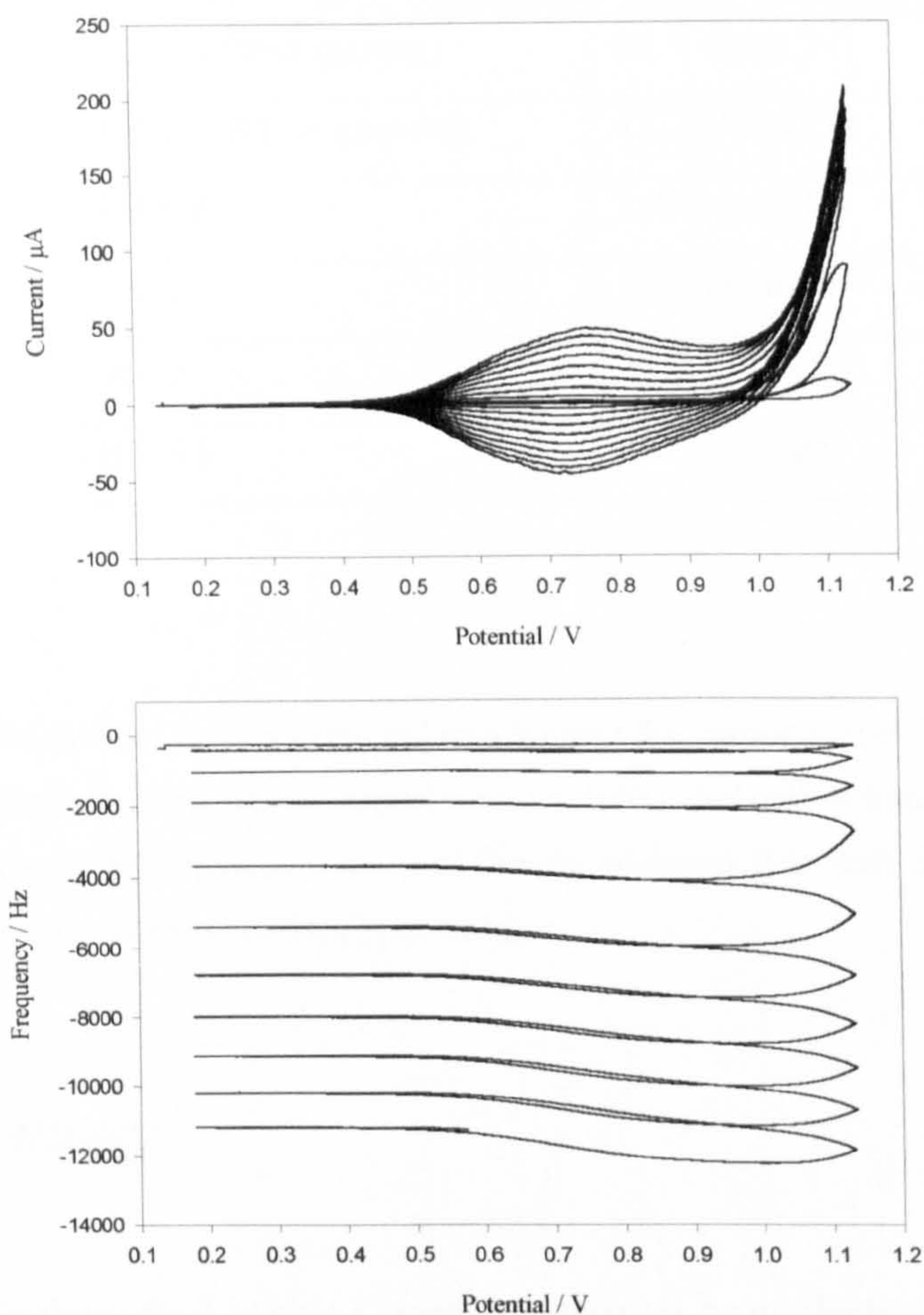
Further investigations were performed to establish if the increase in hydrophilicity of the copolymer films could have an effect on the degree of reaction/immobilisation of reactive species and are discussed in Chapter 5, Section 5.2.1.2.

In light of these findings, which showed a dependence of solvation and copolymer composition on the electrochemical deposition conditions when experiments were performed involving the modification of the unreacted polymer, care was taken to ensure that the same deposition rate and film thickness was always used.



**Figure 2.23:** CV and corresponding QCM crystal frequency changes (mass increase) of the polymerisation of poly(PFP) for quartz crystal electrode immersed in 20 mM PFP, 0.1 M TEAP and acetonitrile vs. Ag|AgCl reference electrode. The voltage was scanned from 0.2 V to 1.05 V using a scan rate of  $20 \text{ mVs}^{-1}$  and electrode area of  $0.229 \text{ cm}^2$ .





**Figure 2.24:** Cyclic voltammogram and corresponding QCM crystal frequency changes (mass increase) of the polymerisation of poly(PFP) for quartz crystal electrode immersed in 20 mM PFP, 0.1 M TEAP and acetonitrile vs. Ag|AgCl reference electrode. The voltage was scanned from 0.2 V to 1.12 V at a scan rate of  $20 \text{ mVs}^{-1}$  and electrode area was  $0.229 \text{ cm}^2$ .

PFP: Py ratio	Solvent volume fraction in as deposited polymer %
100: 0 (fast grown)	64.7 (MeCN)
100: 0 (slow grown)	47.2 (MeCN)
100: 0	3.4 (water)
98: 2	14.1 (water)
95: 5	20.3 (water)
85: 15	26.7 (water)

**Table 2.1:** Estimation of the solvent volume fraction for homopolymer films immersed in MeCN and copolymers immersed in water (experimental results). Solvent volume fraction was calculated by comparing the QCM determined mass of the dry undoped film with the mass of the undoped film when immersed in either pure MeCN or water.

## 2.4 Summary

The results described in this Chapter illustrates how electrochemical techniques were used to prepare poly(PFP) homopolymers and poly(Py/PFP) copolymers used for the research described in the following chapters. It is important that the same growth parameters are used when preparing these polymers, as any major variances can lead to a change in polymer microstructure and film thickness.

In addition, these results show a brief overview of how EQCM techniques can be used as a real time probe for any mass changes that occur on a quartz crystal electrodes are included. The examples mentioned within this Chapter demonstrate that mass changes may occur as a result of the deposition of polymer on the electrode, further modification of the polymer and any changes of solvation properties of copolymer films.



## 2.5 References

1. Pickett, C. J., Ryder, K. S., "Bioinorganic Reaction Centres on Electrodes. Modified Electrodes possessing Amino Acid, Peptide and Ferredoxin-type Groups on a Poly(pyrrole) Backbone", *Journal of Chemical Society, Dalton Transaction*, pp. 2181, 1994.
2. Rai-Choudhury, P. Ed. Handbook of Microlithography, Micromachining, and Microfabrication: Volume 2: Micromachining and Microfabrication, SPIE, 1999.
3. Skotheim, T. A., Elsenbaumer, R. L., Reynolds, J. R., "Electrochemistry of Conducting Polymers" in, "Handbook of Conducting Polymers", Mercel Dekker, New York, 1998.
4. Pletcher, D., A First Course in Electrode Processes. 1st edition, Alresford: Alresford Press Ltd, pp.1-271 1991.
5. Kissinger, P.T., and W.R. Heineman, "Cyclic Voltammetry", *Journal of Chemical Education*, vol. 60, no. 9, pp. 702-706, 1983. .
6. Willard, H. H., Merritt, L. L., Dean, J. A., Settle, F. A., Instrumental Methods of Analysis, Sixth Edition, Wadsworth Publishing Company, 1981.
7. [http://orion1.paisley.ac.uk/macro/Enzyme\\_Electrode/Chapter1/Cyclic\\_Voltammetry1.htm](http://orion1.paisley.ac.uk/macro/Enzyme_Electrode/Chapter1/Cyclic_Voltammetry1.htm).
8. Glidle, A., Swann, M. J., Hadyoon, C. S., Cui, L., Davis, J., Ryder, K. S., Cooper, J. M., "XPS assaying of electrodeposited copolymer composition to optimise sensor materials", *Journal of Electron Spectroscopy and Related Phenomena*, vol. 121, no. 1-3, pp. 131, 2001.
9. Bond, A.M., "Past, present and future contributions of microelectrodes to analytical studies employing voltammetric detection", *Analyst*, vol. 119, pp. R1-R21, 1994.

10. Bruckenstein, S. and M. Shay, "Experimental aspects of use of the quartz crystal microbalance in solution", *Electrochimica Acta*, vol. 30, no.10, pp. 1295, 1985.
11. Nabauer, A., Berg, P., Ruge, I., Muller, E., Woiass, P., Kosslinger, C., Drobe, H., "Biosensors based on piezoelectric crystals", *Sensors and Actuators B*, vol. 1, pp. 508, 1990.
12. Skotheim, T. A., Elsenbaumer, R. L., Reynolds, J. R., "Electrochemistry of Conducting Polymers" in, "Handbook of Conducting Polymers", Mercel Dekker, New York, pp. 542, 1998.
13. Glidle, A., Hadyoon, C. S., Cass, A. E. G., Cooper, J. M., "Modifications of electrodeposited polymers by ion chelation to produce templates for biomolecule immobilisation", *Electrochimica Acta*, vol. 45, no. 22, pp. 3823, 2000.
14. Skotheim, T. A., Elsenbaumer, R. L., Reynolds, J. R., "Superconductor Assemblies and Devices" in, "Handbook of Conducting Polymers", Mercel Dekker, New York, pp. 1029, 1998.
15. Glidle, A., Bailey, L., Hadyoon, C. S., Hillman, A. R., Jackson, A., Ryder, K. S., Saville, P. M., Swann, M. J., Webster, J. R. P., Wilson, R. W., Cooper, J. M., "Temporal and spatial profiling of the modification of an electroactive polymeric interface using neutron reflectivity", *Analytical Chemistry*, vol. 73, no. 22, pp. 5596, 2001.



# Chapter 3

## *Spectroscopic Methods used for the Development and Characterisation of a Polymer System*

### *3.0 Introduction*

A distinct advantage when characterising the PFP polymer matrix is the presence of strong spectroscopic signatures that allow for the ready determination of species' immobilisation using XPS and RAIRS methods (as described in Results section 3.4). Whilst XPS only probes the surface of the polymer, infrared methods can be used to assay the bulk of the film. These complementary analyses have been used to characterise both the unreacted and modified polymer films. This section provides the theory behind both techniques and presents some specific example of how they are used to characterise unmodified and modified PFP based polymers.

Although the homopolymer of PFP is highly reactive in non-aqueous solvents, its hydrophobicity inhibits the reaction with species contained in aqueous solutions. Copolymerisation of PFP with pyrrole was therefore explored and characterised in order to alter the solvation and reactivity of thin poly(PFP) films. It was found that the hydrophilicity of the polymer membrane could be increased by copolymerisation with pyrrole monomers.<sup>1,2</sup> This facilitates penetration of aqueous solvated species throughout the polymer matrix. Preliminary investigations involving reactions with various amine species are presented later (Chapter 5).

In addition the incorporation of Py within the polymer matrix will spatially separate the potential immobilisation sites to allow the attachment of large biomolecules and will possibly minimise steric interactions. One other reason for the production of copolymers was to increase the possible amount of solvent penetration within the film. In Sections 3.4, it is described how XPS and RAIRS measurements can be used to determine the proportion of PFP and Py monomers in the prepared copolymer films.

### ***3.1 Materials***

The materials described in Chapter 2, Section 2.1 were used to prepare the homopolymers and copolymers for XPS measurements. The following materials were also used for XPS imaging experiments:

**Solvents** DMSO (99+%, anhydrous,  $(\text{CH}_3)_2\text{SO}$ ) and MeCN (99.5+%, ACS reagent,  $\text{CH}_3\text{CN}$ ) were obtained from Aldrich.

**Microperoxidase-11** obtained from Sigma/Aldrich.

**Ferrocene Ethylamine** synthesis and structure is described in Chapter 4 (Materials Section 4.1).

**TEAP, PFP, Pyrrole and electrodes** were prepared as described in Chapter 2 (Materials Section 2.1)

## ***3.2 Spectroscopic Methods***

### **3.2.1 X-ray Photoelectron Spectroscopy (XPS)**

#### **3.2.1.1 Background**

A widely used spectroscopic technique for surface characterisation is X-ray Photoelectron Spectroscopy (XPS) also known as Electron Spectroscopy for Surface Analysis (ESCA). This technique employs x-rays to detach electrons from the



surface under investigation. The difference in energy between the incident x-rays ( $h\nu$ ) and the kinetic energy of the emitted electrons ( $E_K$ ) is equivalent to the binding energy of the electron. When the sample to be examined is placed in a high vacuum and irradiated with photons of sufficiently high energy (in the x-ray energy range), the atoms on the surface emit photoelectrons after direct transfer of energy from the photon to the core-level electron. The emitted electrons are subsequently detected by an electron spectrometer, which separates them according to their kinetic energy and displays them on a digital screen as element-specific spectra. The basic concept and a schematic diagram of the XPS apparatus are shown in Figure 3.1 and Figure 3.2.

The analyser system used in XPS consists of three components: the collection lens, the energy analyser and the detector. The lens system can collect photoelectrons over a  $30^\circ$  angle. The higher the collection angles the higher the number of photoelectrons collected per incident x-ray.

In addition to collecting the photoelectrons, the lens system on most spectrometers retards their kinetic energy. The entire XPS spectrum is acquired by ramping appropriate voltages on the different lens elements. The lens system also projects the analysed area a distance away from the energy analyser, which allows the sample to be positioned so that it is more readily accessible to the X-ray source and other components in the vacuum system.

The most common energy analyser used in XPS experiments is the electrostatic hemispherical analyser. It consists of two concentric hemispheres. A potential is placed across the hemispheres such that the outer hemisphere is negative and the inner hemisphere is positive with respect to the potential at the centre line. The potential at the centre line is called the pass energy.

Most XPS experiments are done with a constant pass energy. This will maintain a constant absolute resolution,  $\Delta E$ , for all photoelectron peaks. Since the analyser resolution is defined as  $\Delta E/E$ , where  $E$  is the energy of the electron as it passes through the analyser. The ratio is a constant for any given analyser, so if  $E$  is fixed (constant pass energy),  $\Delta E$  will also be constant.

Hemispherical analysers are classified as dispersive analysers, that is the electrons are deflected by an electrostatic field. There is a range of electron energies that can successfully travel from the entrance to the exit of the analyser without undergoing a collision with one of the hemispheres. The magnitude of this electron energy range depends on quantities such as the pass energy, the size of entrance slits and the angle with which the electrons enter the analyser.

The electrons are counted once they have passed through the energy analyser. Since electrons arrive at the analyser exit with a range of energies, the most efficient means of detection is the multi-channel array to count the number of electrons leaving the analyser at each energy. One method of accomplishing this is to use a channel plate to magnify the electron current and a resistive strip anode to monitor the position, and therefore, the energy of the electrons.

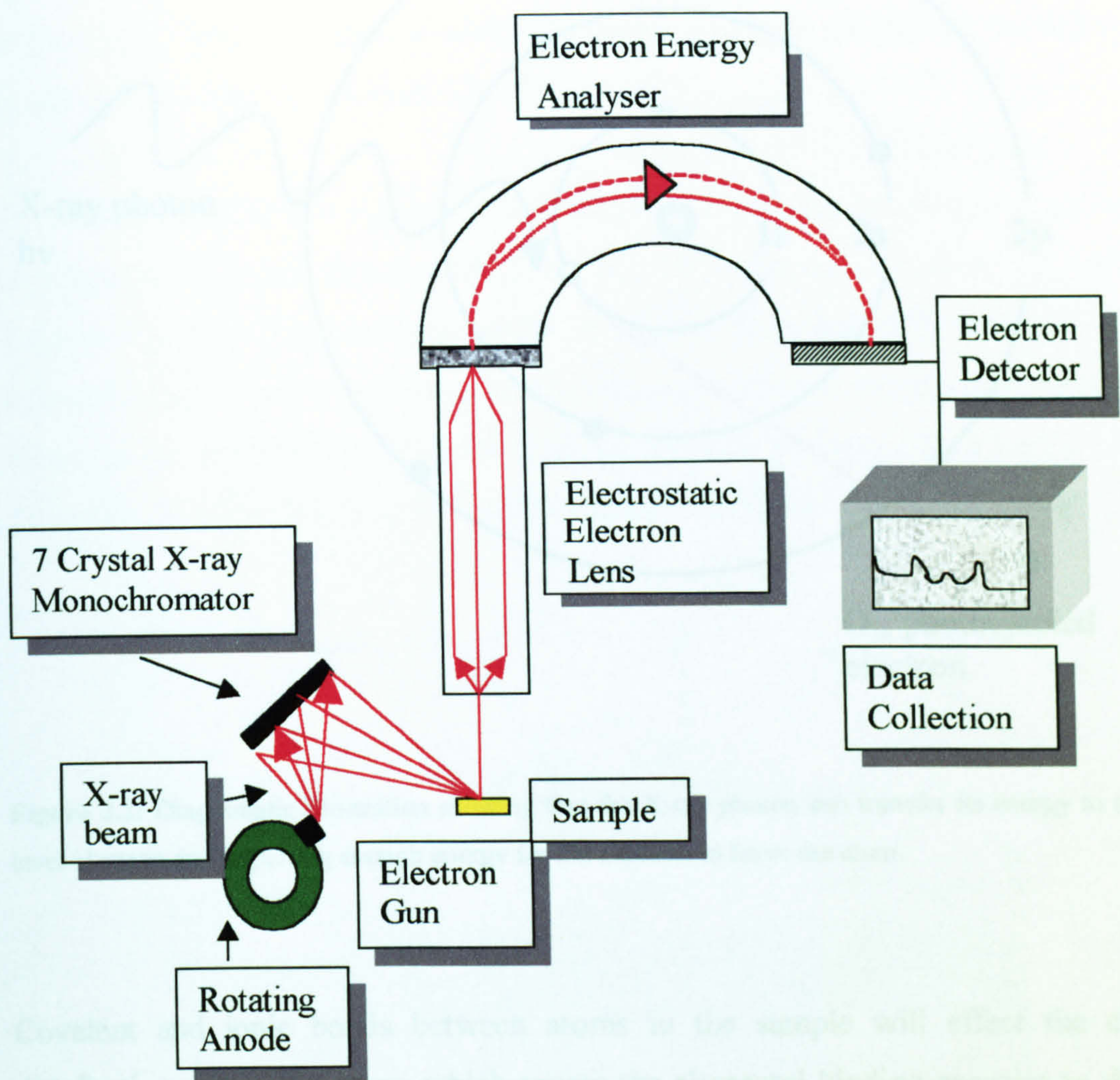
The measured kinetic energy with which the photoelectrons are emitted from the surface region can be described by Equation:

$$E_K = h\nu - E_B - \phi \quad \text{(Equation 3.1)}$$

Where  $\phi$  is the spectrometer work function (a constant for a given analyser). The ability to accurately measure the kinetic energies of the photoelectrons permits the determination of their binding energies,  $E_B$ , which thereby provides valuable chemical information on the surface configuration. The detector counts the number of photoelectrons at the individual set of binding energies, which allows discrete differentiation of the components on the surface of the sample.

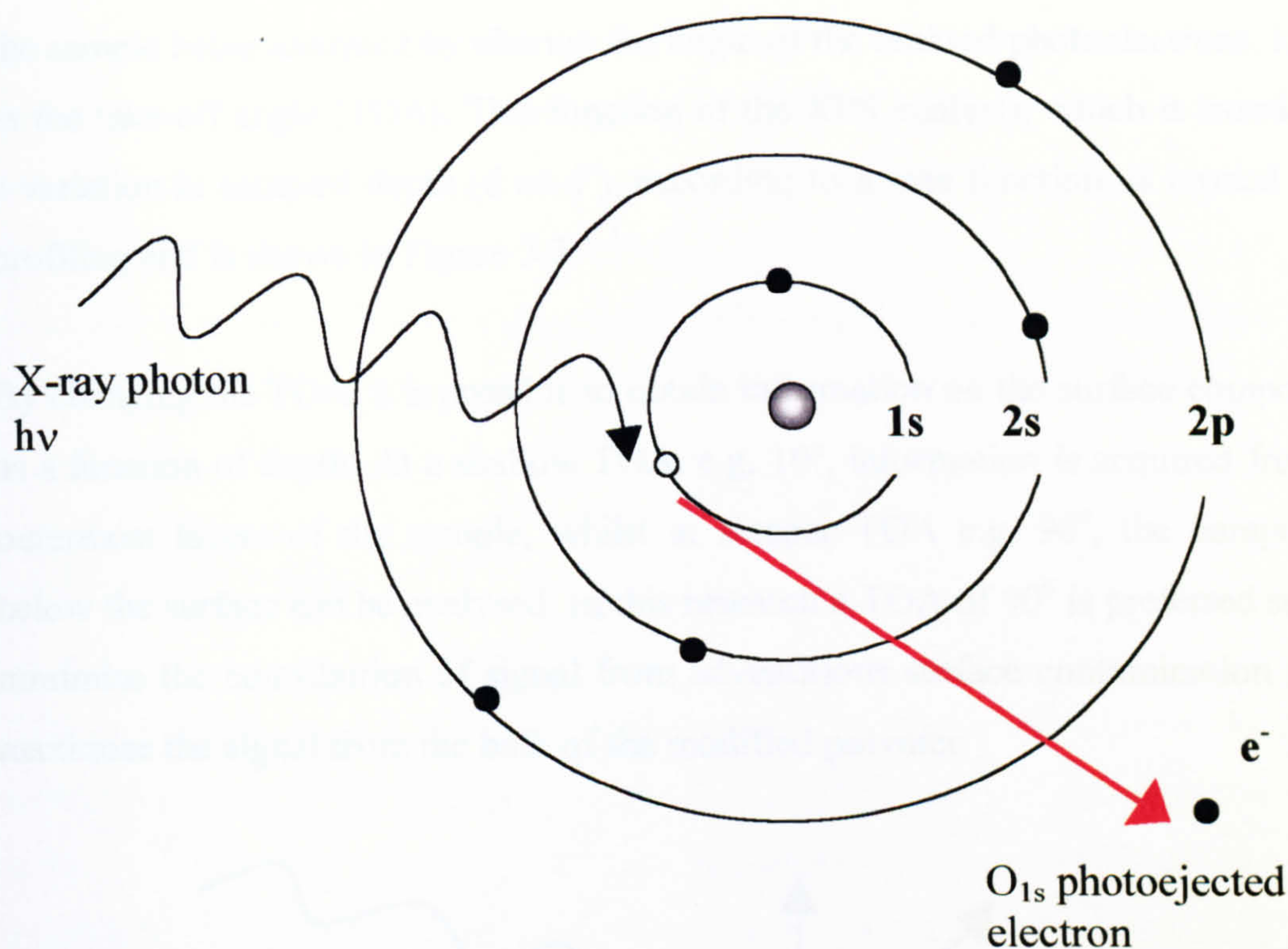
Two types of electrons are emitted; inner core and the more loosely held valence electrons. When the photon ejects the inner-core electron (photoionization), the effective increase in nuclear charge causes a reorganisation of the valence electrons from an occupied to an unoccupied level (a process called shake-up).





**Figure 3.1:** A schematic diagram of an XPS spectrometer. The key components (a X-ray anode, monochromator crystal, collection lens, hemispherical analyser and a large area detector) are shown.





**Figure 3.2:** Diagrammatic illustration showing that the X-ray photon can transfer its energy to a core-level electron and imparting enough energy for the electron to leave the atom.

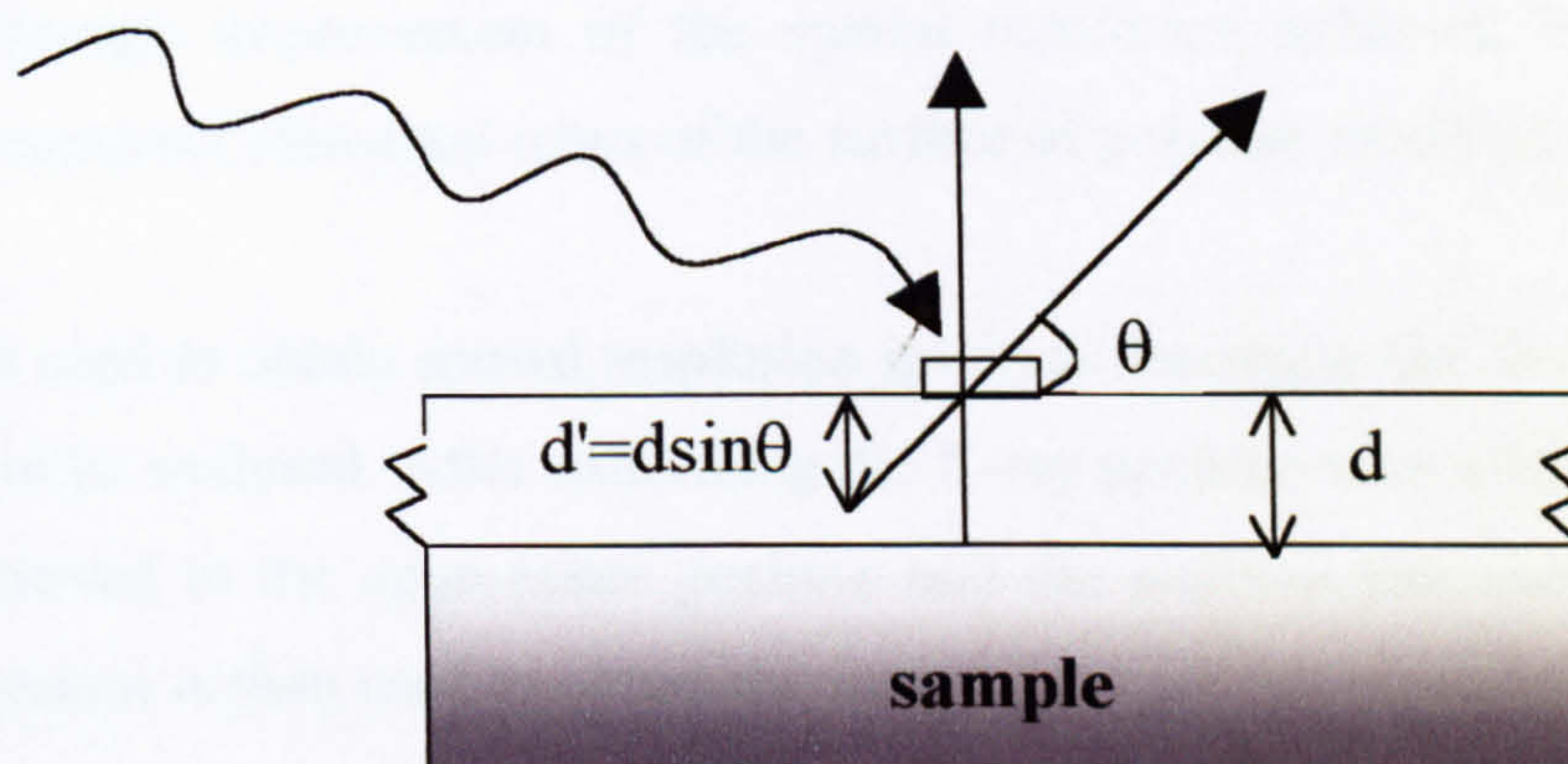
Covalent and ionic bonds between atoms in the sample will effect the charge distribution within the atom, which causes the elemental binding energies to shift in relation to the free neutral atom. The size of this shift is dependent upon the functional group bound to the atom, allowing identification of specific bond types.<sup>3-6</sup> This will be described in more detail later in this Chapter. These chemical shifts can be observed for every element in the periodic table, with the exception of hydrogen, but it is the applicability to the second row elements including carbon, nitrogen and oxygen that makes XPS such an important tool for bio-organic interfaces.

The XPS spectra obtained from the experiments provide information only on the atomic species that are present within approximately 10 nm of the sample surface. Although the incident X-rays can penetrate through the sample to extensive depths, the escape length is determined by the kinetic energy of the emitted photoelectron. Only those electrons in close proximity to the surface will be able to sustain sufficient energy to escape. Within this distance it is possible to change the depth of



the sample being analysed by altering the angle of the emitted photoelectrons, known as the take-off angle (TOA). This function of the XPS analysis, which is based upon a variation in sampled depth ( $d$  or  $d'$ ), according to a sine function, is termed depth profiling and is shown in Figure 3.3.<sup>3,5</sup>

By changing the TOA, it is possible to obtain information on the surface composition as a function of depth. At a shallow TOA, e.g.  $10^\circ$ , information is acquired from the outermost layers of the sample, whilst at steeper TOA e.g.  $90^\circ$ , the composition below the surface can be analysed. In this research a TOA of  $90^\circ$  is preferred so as to minimise the contribution of signal from adventitious surface contamination and to maximise the signal from the bulk of the modified polymer.



**Figure 3.3:** Schematic diagram showing the effect of varying the take-off angle of photoelectrons on the depth of penetration into the sample and, hence, the information about sample composition.

When examining samples using XPS it is usual to initially take a spectrum over a wide range of energies (a survey spectrum) before performing detailed analyses on the particular areas of interest for the individual sample. To allow proper measurement of the  $E_B$ , a calibrated and suitably referenced ESCA spectrometer is required. For conducting samples (such as metals) the sample can be placed in electrical contact with the spectrometer and calibrated so that  $E_B$  values can be obtained.<sup>18</sup> However, some materials do not have sufficient electrical conductivity or cannot be mounted in electrical contact with the ESCA spectrometer. These samples require an additional source of electrons to compensate for the positive charge built up by the emission of the photoelectrons. This is accomplished by flooding the



sample with a monoenergetic source of low-energy (<20 eV) electrons. Furthermore, an internal reference is used for calibration. Typically for polymers and organic samples the hydrocarbon component of a C(1s) peak is set at 285 eV. A flood gun was used in this research while analysing the samples as the underlying electrode was gold evaporated on glass (insulated). All spectra illustrated in this thesis have been referenced to C (1s) peak at 285 eV.

### **3.2.1.2 XPS Imaging<sup>7-10</sup>**

XPS systems can also be used to image chemical features with spatial detail. The application of XPS can be used to analyse the distribution of chemical features at surfaces. Through improvement of the spatial resolution achieved by XPS it is possible to construct elemental maps of the surface of polymer modified electrodes.

The method used to obtain spatial resolution involves focussing the X-ray source on the sample to be analysed. After calibrating the X-ray position with a microscope, the sample is moved to the appropriate position and the analysis can start. A position sensitive detector is then used to obtain the image.

## **3.2.2 Reflectance-Adsorption Infra Red Spectroscopy (RAIRS)**

### **3.2.1.1 Background**

RAIRS is an analytical method, which can be used in conjunction with XPS to obtain complimentary and/or corroborative information regarding the composition of the bulk of the sample. This section describes the theory behind RAIRS spectroscopy and why it is a useful probe for polymer characterisation.

RAIRS involves light from an infrared source being passed through a sample of an organic compound. Some wavelengths will then be absorbed, whilst others are transmitted without absorption. Particular functional groups in the sample absorb light of known wavelengths (or energy) to produce characteristic infrared spectrum. When describing the appearance of different absorption bands in the spectra it is



important to consider the fundamental molecular movements associated with chemical bonds. Chemical bonds are able to undergo a variety of movements such as stretching, bending, twisting and rotating. The first two of these vibrational changes within the molecules absorb energy within the infrared region of the electromagnetic spectrum.

Each bond within a continuously vibrating organic molecule has its own characteristic frequency arising from both stretching and bending vibrations. The stretching frequencies are due to vibrational changes in bond length, whilst the bending frequencies involve vibrational changes in bond angle. A greater amount of energy is required to stretch the bond between two atoms than to bend it, and, as a consequence, the stretching absorptions for the same bond increases. In addition, the vibrational frequency of a bond increases when the bond strength increases and the mass of either of the atoms involved in the bond decreases. For example, the frequency associated with an alkene (C=C) and a carbonyl (C=O) group stretches are higher than those for an alkane (C-C) and an alcohol (C-O) group stretches respectively.<sup>11</sup>

The RAIRS technique that is used in this research is reflectance-absorption infra-red spectroscopy (RAIRS or FT-RAIRS). This has been demonstrated to be a powerful technique for the characterisation of adsorbates and conducting films on metallic surface<sup>12</sup>. The radiation is split into s and p components, where p refers to parallel polarised radiation and s to perpendicular polarised radiation. It has been demonstrated in reflectance measurements that the absorption of IR radiation involves only the p polarised IR beam. In this case, p -polarised radiation (parallel) possesses the ability to interact with the sample<sup>13</sup>. Furthermore, the only active vibrations that may be observed using RAIRS are those that have a component of the dipole polarised in the direction normal to the surface plane. This is called the 'surface selection rule' for reflectance IR spectroscopy.

The intensity of the absorption bands is dependent upon the magnitude of the dipole change during the vibration<sup>14</sup>: the larger the change, the stronger the absorption band. The band intensity of a particular functional group also depends on the number of



groups present in the sample being studied. For example groups such as C=O, C-F have stronger bands than C-C groups.

### ***3.3 Results and Discussion***

#### **3.3.1 XPS Analysis of PFP Homopolymers**

The polymers that were used for spectroscopic analysis were deposited on a 1 cm<sup>2</sup> Au macroelectrode and the samples were used for both XPS and IR analysis. Since the electrode area was 1cm<sup>2</sup>, this made them sufficiently large enough to obtain relevant information on the surface composition. To keep the surface contamination to a minimum, all samples were stored in a desiccator immediately after preparation. XPS analysis of each sample was performed by firstly taking a survey scan spectrum covering a range between approximately 0 eV and 100 eV. High-resolution spectra were collected F (1s), C (1s), N (1s) and O (1s) regions (as illustrated in Figure 3.4).

The binding energy of peaks seen in the C (1s) and O (1s) spectra depend on the initial state of the element. The initial state is the ground state of the atom prior to photoemission. By formation of chemical bonds with other atoms the  $E_B$  (Equation 3.1) of the electrons in that atom will change. The change in  $E_B$  is called the chemical shift. As the formal oxidation states of an element increases or the local electron density increases, the  $E_B$  of the photoelectrons ejected from that element will increase. When taking into consideration the C (1s) spectra of the PFP polymer system, the carbons present have various chemical environments. These include C-H < C-C < C=C < C-F < O=C-O. The  $E_B$  of these carbons increase in the indicated order and are consistent with the initial state effect. In addition, the oxygen  $E_B$  values also depend on the chemical environment where functional group may be present.

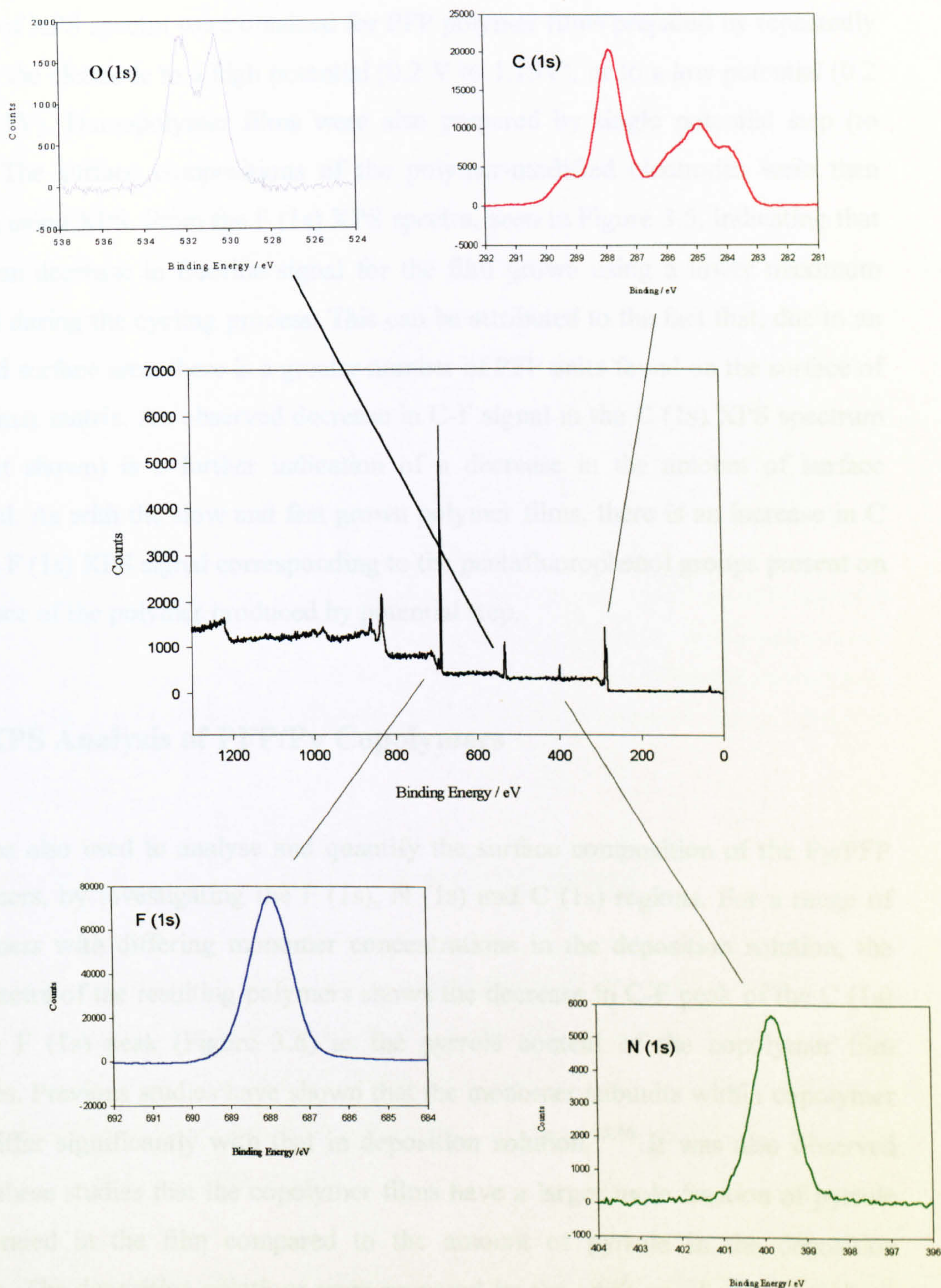
Furthermore, the areas under the peaks associated with various elements are related to the amount of each element present. By measuring the peak areas and correcting them for the appropriate instrumental factors and sensitivity, the percentage of each element on the surface can be determined and used to calculate elemental ratios. For the PFP homopolymer the calculated elemental ratios of C :N :F :O were 13: 1: 5 :2 which would be expected when considering the chemical structure of the PFP



monomer unit. The energy scales of the spectra presented were corrected to the major C-H (1s) peak at 285 eV (or the N (1s) peak at 400 eV when it was difficult to isolate the major C-H peak).

When probing the extent of poly(PFP) with selected nucleophiles each sample was analysed by both XPS and RAIRS spectroscopy before and after reaction to measure the extent of reaction.





**Figure 3.4:** High resolution O (1s), C (1s), F (1s) and N (1s) spectra and survey XPS spectra (black) of homopolymer of PFP (TOA 90 °). The homopolymer modified 1 cm<sup>2</sup> electrode was prepared by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN.



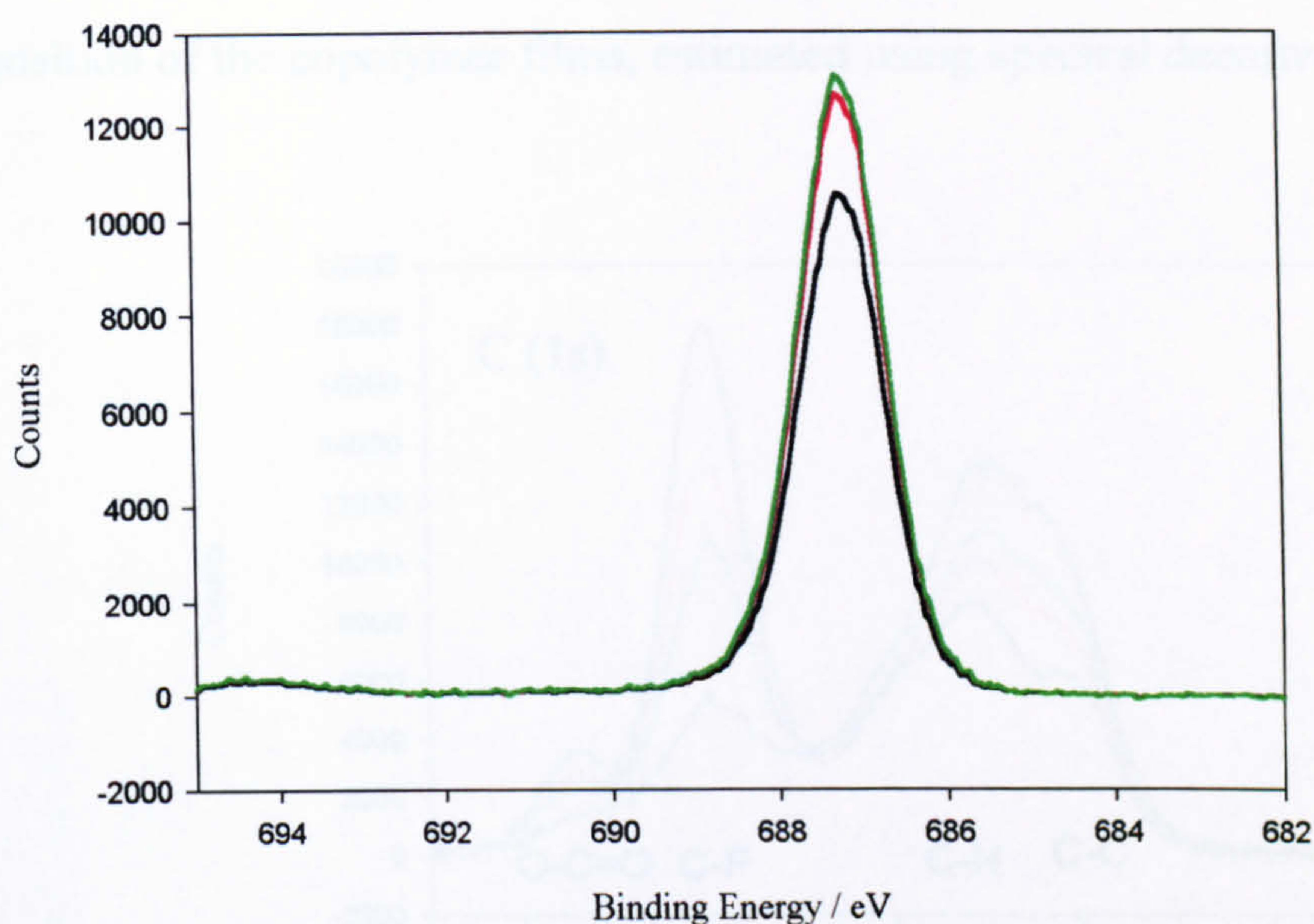
### **3.3.1.1 Comparison of Homopolymers Prepared Using Different Electrochemical Techniques**

A series of XPS spectra were obtained for PFP polymer films prepared by repeatedly scanning the electrode to a high potential (0.2 V to 1.15V), or to a low potential (0.2 V to 1.11V). Homopolymer films were also prepared by single potential step (to 1.15V). The surface compositions of the polymer-modified electrodes were then analysed using XPS. From the F (1s) XPS spectra, seen in Figure 3.5, indicating that there is an decrease in fluorine signal for the film grown using a lower maximum potential during the cycling process. This can be attributed to the fact that, due to an increased surface area, there is a greater number of PFP units found on the surface of the polymer matrix. An observed decrease in C-F signal in the C (1s) XPS spectrum (data not shown) is a further indication of a decrease in the amount of surface measured. As with the slow and fast grown polymer films, there is an increase in C (1s) and F (1s) XPS signal corresponding to the pentafluorophenol groups present on the surface of the polymer produced by potential step.

### **3.3.2 XPS Analysis of PFP/Py Copolymers**

XPS was also used to analyse and quantify the surface composition of the Py/PFP copolymers, by investigating the F (1s), N (1s) and C (1s) regions. For a range of copolymers with differing monomer concentrations in the deposition solution, the XPS spectra of the resulting polymers shows the decrease in C-F peak of the C (1s) and the F (1s) peak (Figure 3.6) as the pyrrole content of the copolymer film increases. Previous studies have shown that the monomer subunits within copolymer films differ significantly with that in deposition solution.<sup>15,16</sup> It was also observed during these studies that the copolymer films have a larger mole fraction of pyrrole incorporated in the film compared to the amount of pyrrole in the deposition solution. The deposition solutions were prepared by the addition of a small volume of pyrrole and acetonitrile solution to the PFP monomer, acetonitrile and TEAP solution (as described in Chapter 2, Section 2.3.2).





**Figure 3.5:** F (1s) XPS spectra (TOA 90 degrees) unreacted homopolymer of PFP grown by scanning potential to 1.11 V (black line), grown by scanning potential to 1.15V (red line) and by potential step to 1.15 V for 60 seconds (green line). Both films grown on 1 cm<sup>2</sup> electrode by scanning the potential to 1.02 V in a deposition solution of 20 mM PFP, 0.1 M TEAP and acetonitrile vs. Ag|AgCl reference electrode at a scan rate of 20 mVs<sup>-1</sup>.

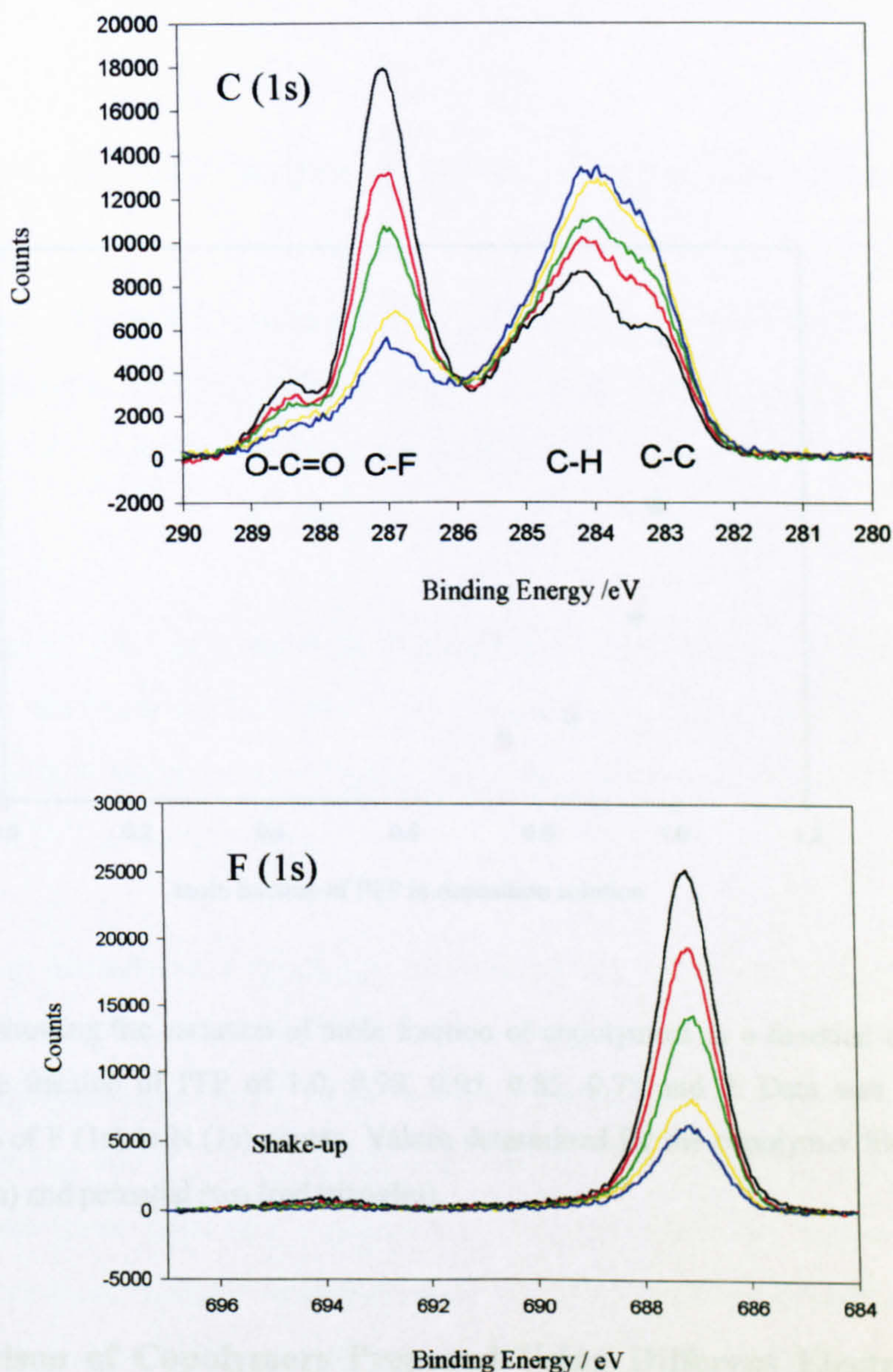
### 3.3.2.1 Methods Used to Establish Copolymer Compositions<sup>17</sup>

XPS analysis was used to determine the composition of the copolymers prepared from solutions containing different ratios of Py and PFP. This was carried out using two different methods. The first method utilised the different line shapes of the C (1s) spectra for polymers deposited from solutions containing the single monomer solutions of Py and PFP respectively.

The difference between the C (1s) envelope of the two library spectra was used to evaluate the composition of the surface of the copolymer films. Spectral deconvolution of the C (1s) XPS signal can provide the mole fraction of the two monomer containing species (PFP and pyrrole) in a given copolymer film.



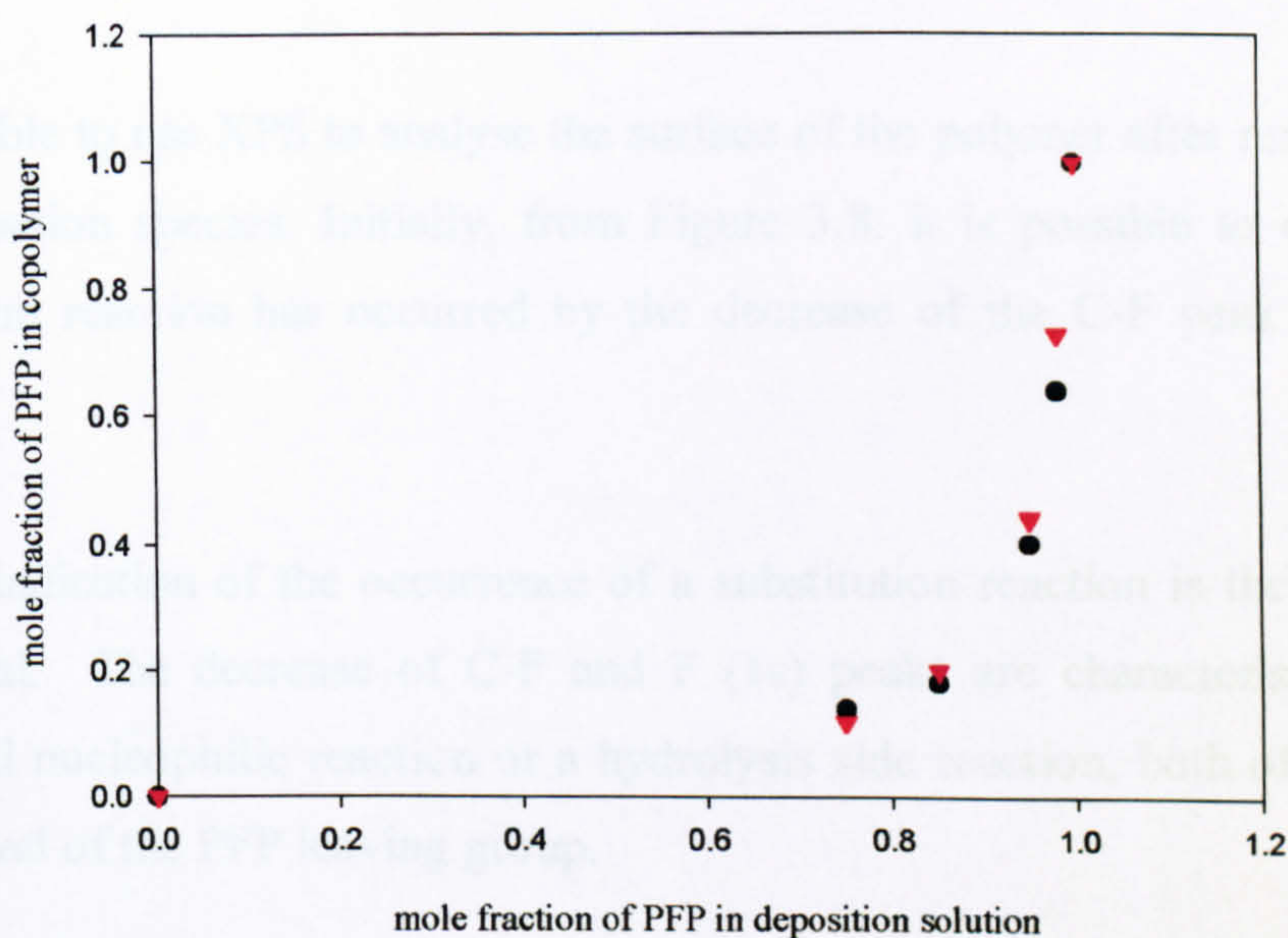
In addition since fluorine is only found in the PFP group whereas nitrogen is in both the PFP and Py monomer units, assessment of the film composition can also be made using the ratio of the F (1s): N (1s) signals. Application of this method corroborated the composition of the copolymer films, estimated using spectral deconvolution.



**Figure 3.6:** C (1s) and F (1s) XPS spectra (TOA 90 degrees) of unreacted copolymers prepared from potential cycling electropolymerisation from solutions containing PFP and Py monomers with a PFP monomer mole fractions of 1.00 (black), 0.98 (red), 0.95 (green), 0.85 (yellow) and 0.75 (blue).



Figure 3.8 shows that there is a rapid decrease in PFP monomer composition following the initial addition of the small quantity of pyrrole monomer to the deposition solution. For example, to prepare a copolymer film with 50:50 ratio of PFP and Py incorporated, the deposition solution requires a mole fraction of 0.96 PFP.



**Figure 3.7:** Graph showing the variation of mole fraction of copolymers as a function of deposition solutions with mole fraction of PFP of 1.0, 0.98, 0.95, 0.85, 0.75 and 0. Data was obtained by calculating the ratio of F (1s) to N (1s) counts. Values determined for the copolymer films produced by CV (black circles) and potential step (red triangles).

### 3.3.2.2 Comparison of Copolymers Prepared Using Different Electrochemical Techniques

Experiments to investigate the influence of electrodeposition conditions on the composition of the resultant copolymer were carried out and, a series of XPS spectra were obtained for Py/PFP copolymer films prepared by repeatedly scanning the electrode potential between 0 V and 1.15V (at 20 mV/s). For deposition solutions with identical compositions, copolymers prepared by potential scanning had a lower



PFP composition when compared to those prepared by single potential step (to 1.15 V) (Figure 3.7). This is a consequence of the proportion of time the electrode potential is above that required for the electrodeposition of Py but below that required for PFP polymerisation. For a given solution, the difference in composition between films prepared using step or scan methods is largest for deposition solutions containing small amounts of pyrrole.

### 3.3.3 Use of XPS for the Analysis of Immobilisation Reactions

It is possible to use XPS to analyse the surface of the polymer after reaction with the immobilisation species. Initially, from Figure 3.8, it is possible to observe that a substitution reaction has occurred by the decrease of the C-F peak in the C (1s) spectra.

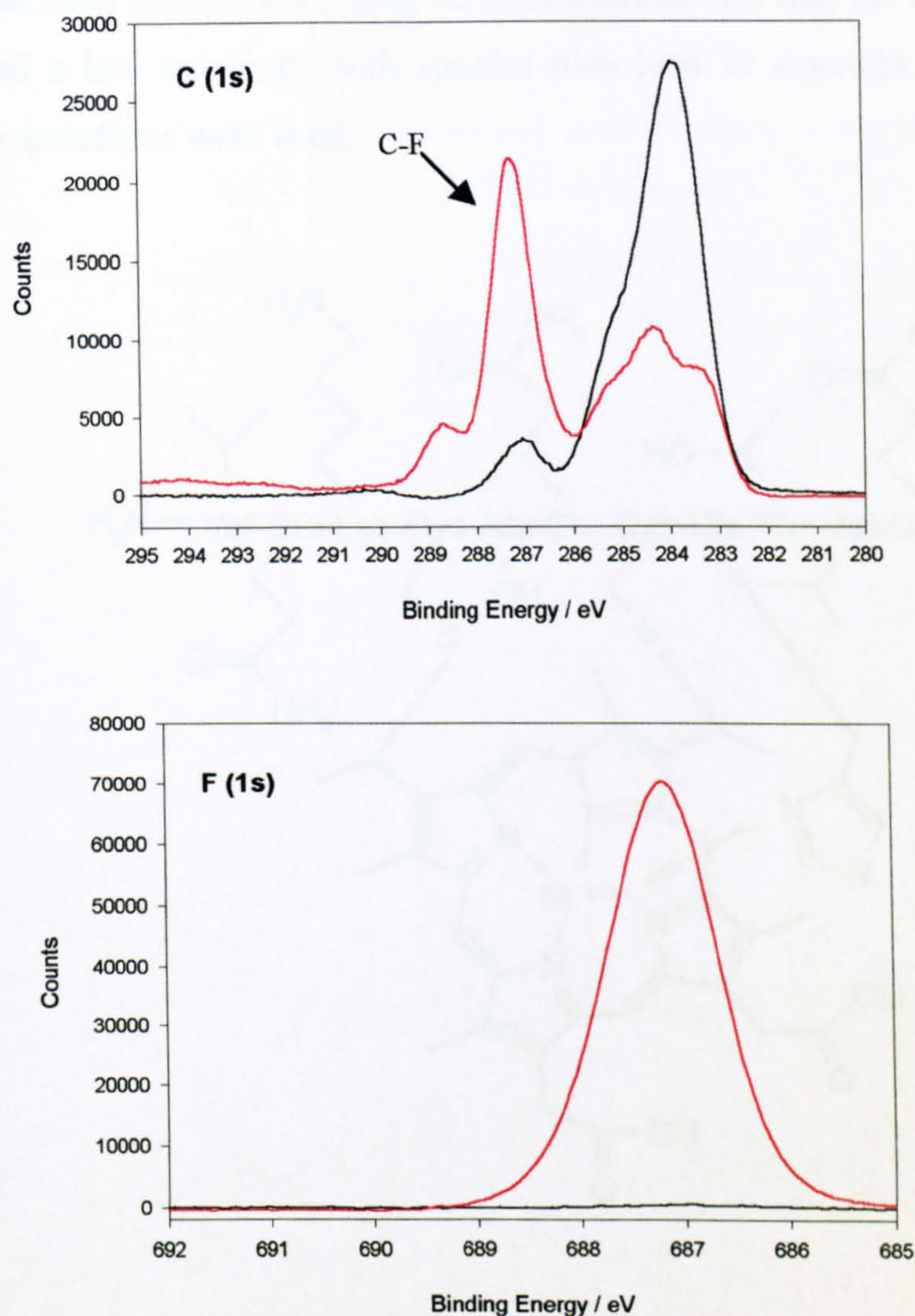
Another indication of the occurrence of a substitution reaction is the decrease of F (1s) signal. The decrease of C-F and F (1s) peaks are characteristic of either a successful nucleophilic reaction or a hydrolysis side reaction, both of which lead to the removal of the PFP leaving group.

To confirm that an immobilised species is present on the surface of the polymer it is useful for it to have a distinctive XPS spectroscopic probe. In further Chapters the majority of the compounds used have such a probe, for example, Fe (2p), in ferrocene ethylamine, and S (2p), in microperoxidase. The biological species used in Chapters 6 do not have naturally occurring probe, therefore, to uniquely identify these biomolecules were labelled with iodine or bromine moieties to make it possible to determine their presence. This labelling technique is discussed in the appropriate chapters.

XPS only measures the interfacial composition of the polymer films (e.g. polymer-solution interface), however, the polymer-electrode interface was exposed by using adhesive tape (sellotape) to peel off the film from the electrode (the tape was analysed by XPS to confirm that only the carbon and oxygen are present; nitrogen, sulphur, halogens etc. are absent). This measurement enables the determination of



immobilisation in regions of the polymer most distant from the solution interface. As may be expected the extent of this immobilisation depends on diffusion through the polymer film. This technique is also used in Chapter 4 (Section 4.2.1.1) to investigate the diffusion of a redox species throughout the polymeric matrix.

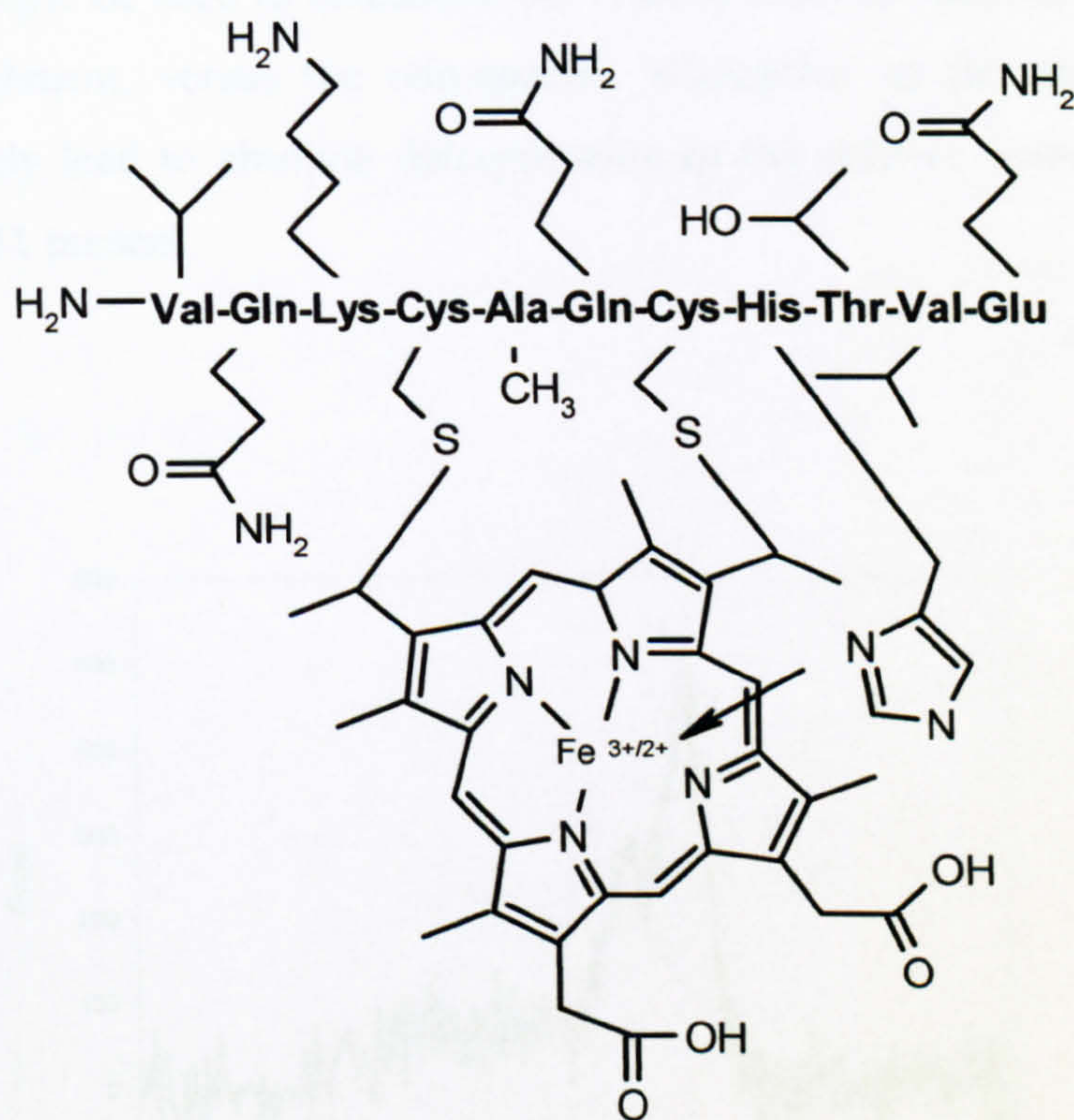


**Figure 3.8:** C (1s) and F (1s) spectra (TOA 90 degrees) of homopolymer of PFP unreacted (red line) and after reaction with an amine species (black line). Note the decrease in peak at 287 eV in the C (1s) spectra, which indicates a decrease in C-F due to the removal of the pentafluorophenyl leaving group.



### 3.3.3.1 XPS to Analyse Immobilisation of Copolymer Interfaces

Experiments to determine whether the immobilisation of a larger biomolecule was possible were carried out. The immobilisation of the microenzyme, MP-11, via a nucleophilic reaction of the terminal amines of the lysine residues with the activated ester sites was attempted (Figure 3.9 shows the sequence of the 11 amino acids). Since it had been established (using XPS measurements) that the homopolymer PFP of PFP had a low reactivity with species dissolved in aqueous solutions, PFP/Py copolymer interfaces were used.

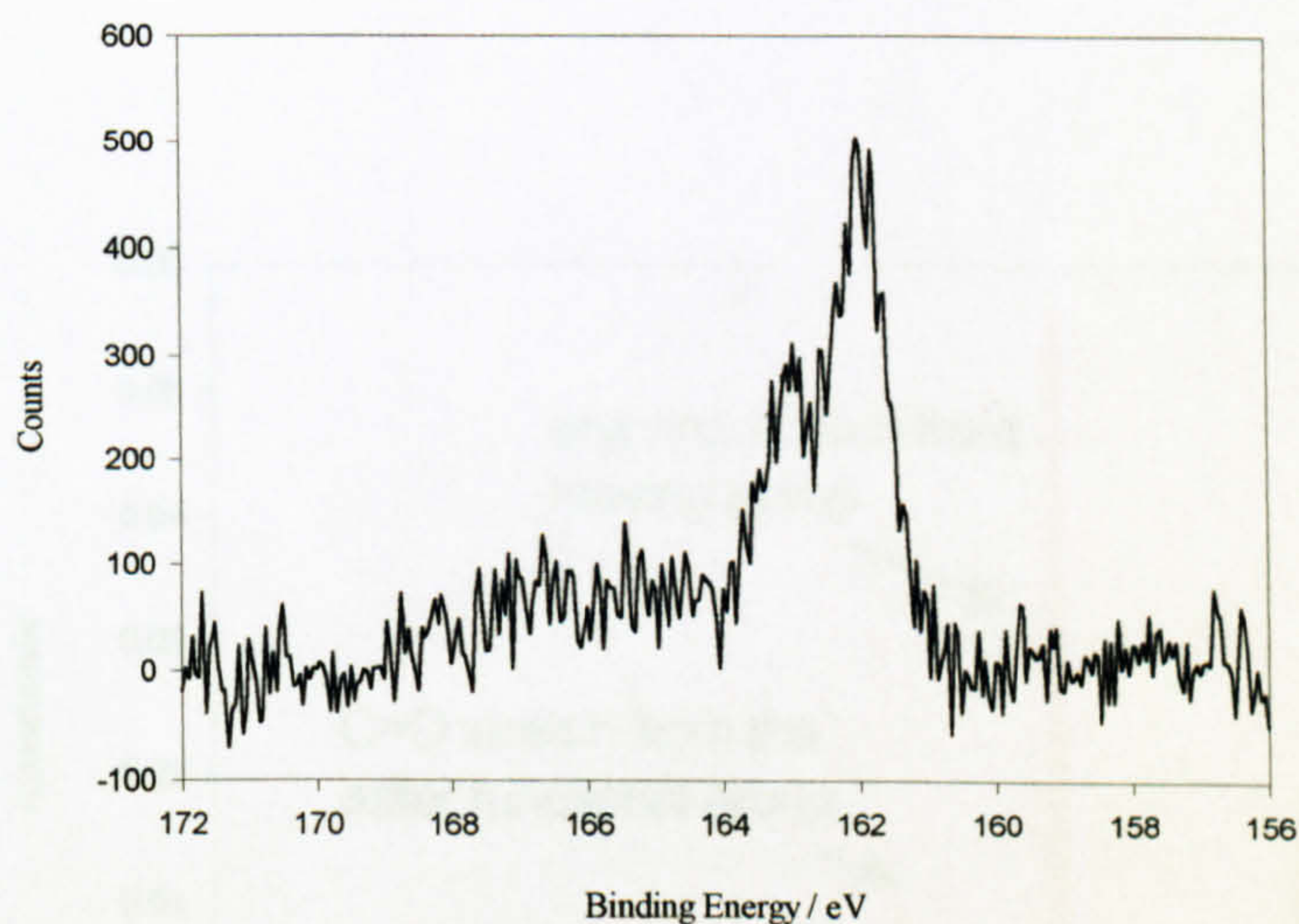


**Figure 3.9:** Schematic diagram of Microperoxidase-11 (with 11 amino acids). Note the presence of the Fe centre of the heme group and the presence of sulphur of cystamine amino acids.

Using XPS, the presence of MP-11 can be determined by measurements in the S (2p) region (arising from the cystamine residues). In addition MP-11 has a Fe (2p) XPS signal due to the presence of the heme bound Fe centre, which presents a further means of identification of microenzyme immobilisation. S (2p) XPS measurements for the poly(PFP) and PFP/Py copolymers after the polymer coated electrodes were



immersed in an aqueous solution of MP-11 and showed that the optimum amount of MP-11 immobilisation occurred with the copolymer film deposited from a solution containing a ratio of 85:15 PFP: Py (Figure 3.10). Furthermore, the control experiments, which involved immersing poly(PFP) and poly(pyrrole) electrodes in the aqueous solution of MP-11 showed that there was no immobilisation of MP-11 on the interface of the homopolymer of PFP. Also, there was a significantly smaller signal on the poly(pyrrole) interface relative to the optimum copolymer signal. The appearance of this small S (2p) signal on a poly(pyrrole) surface is due to non-specific adsorption of MP-11 on the surface of the film. The ratio of the two signals could, in principle be used to determine the relative rates of reaction of the specific, covalent attachment, versus the non-specific adsorption of the biomolecule, and could ultimately lead to absolute determination of the relative amounts of the two forms of MP-11 present.



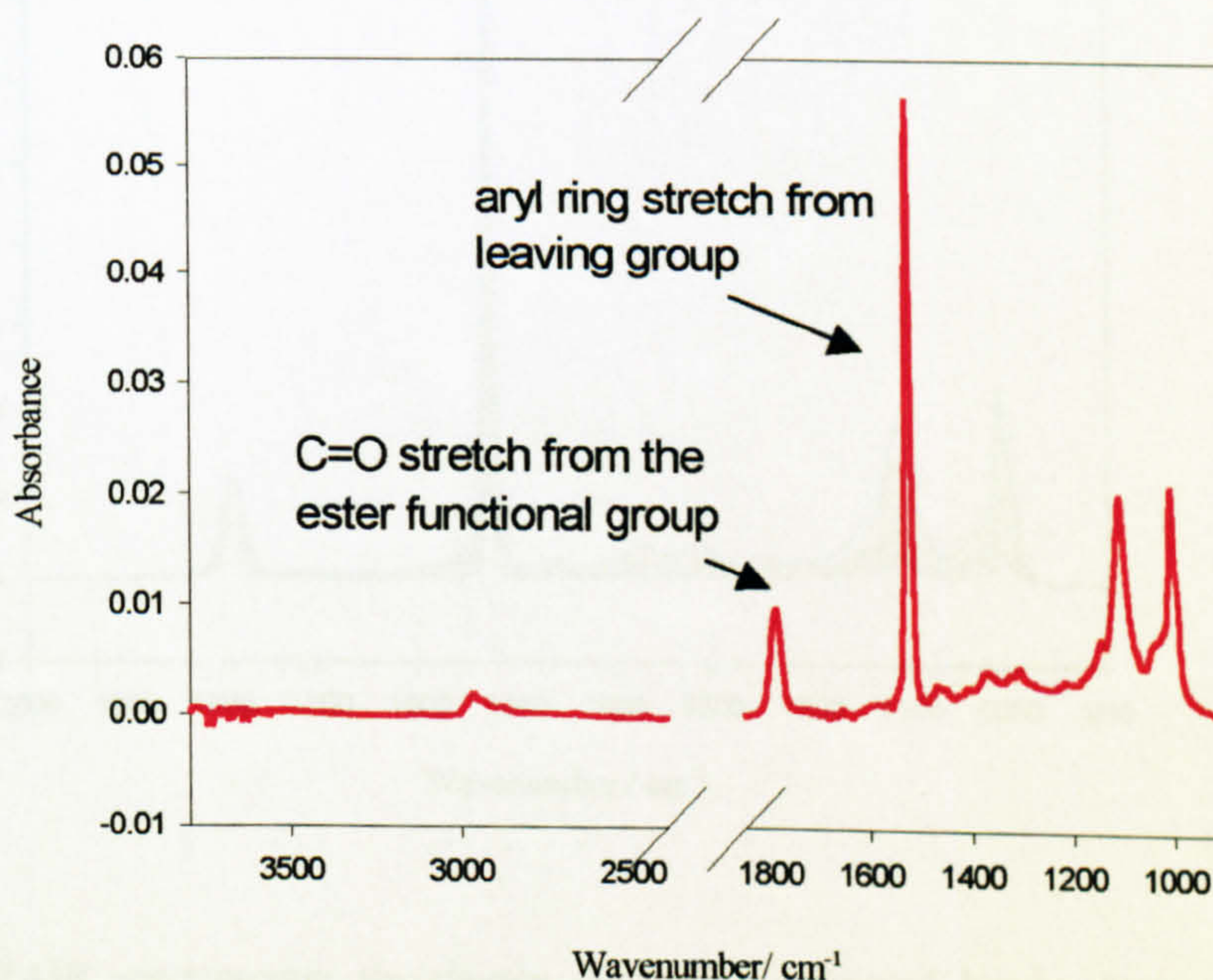
**Figure 3.10:** S (2p) XPS spectra (TOA 90 degrees) of PFP/Py copolymer, polymerised from solution containing PFP and Py monomers with PFP monomer mole fraction of 0.85, after reaction with aqueous solution of MP-11 and pH 8.8 phosphate buffer (pH 8.8 buffer is needed to ensure that the free amine  $\text{NH}_2$  is available to react with the ester).



### 3.3.4 Application of RAIRS Spectroscopy to Homopolymer Analysis

RAIRS was used extensively to characterise of the electrodeposited polymers studied in this work. The use of this technique was facilitated by the high reflectivity of the underlying gold electrodes on which the polymer films were grown (at a RAIRS angle of approximately  $76^\circ$ ). Instrumental limitations (including the detector cut-off and ZnSe polariser) restricted the measurement range to ca  $4000\text{ cm}^{-1}$  to  $1000\text{ cm}^{-1}$ . A typical example of a poly(PFP) spectrum is shown in Figure 3.11.

The data collected was subjected to a degree of data processing, which involved the conversion from reflectivity into absorbance and subsequent normalisation for variations in reference and sample area by shifting the spectra so that the non-adsorbing region between  $2000\text{ cm}^{-1}$  and  $2700\text{ cm}^{-1}$  lay on the absorbance axis at zero. Once the data was in this form, it was possible to compare the peaks on the spectra with other samples.

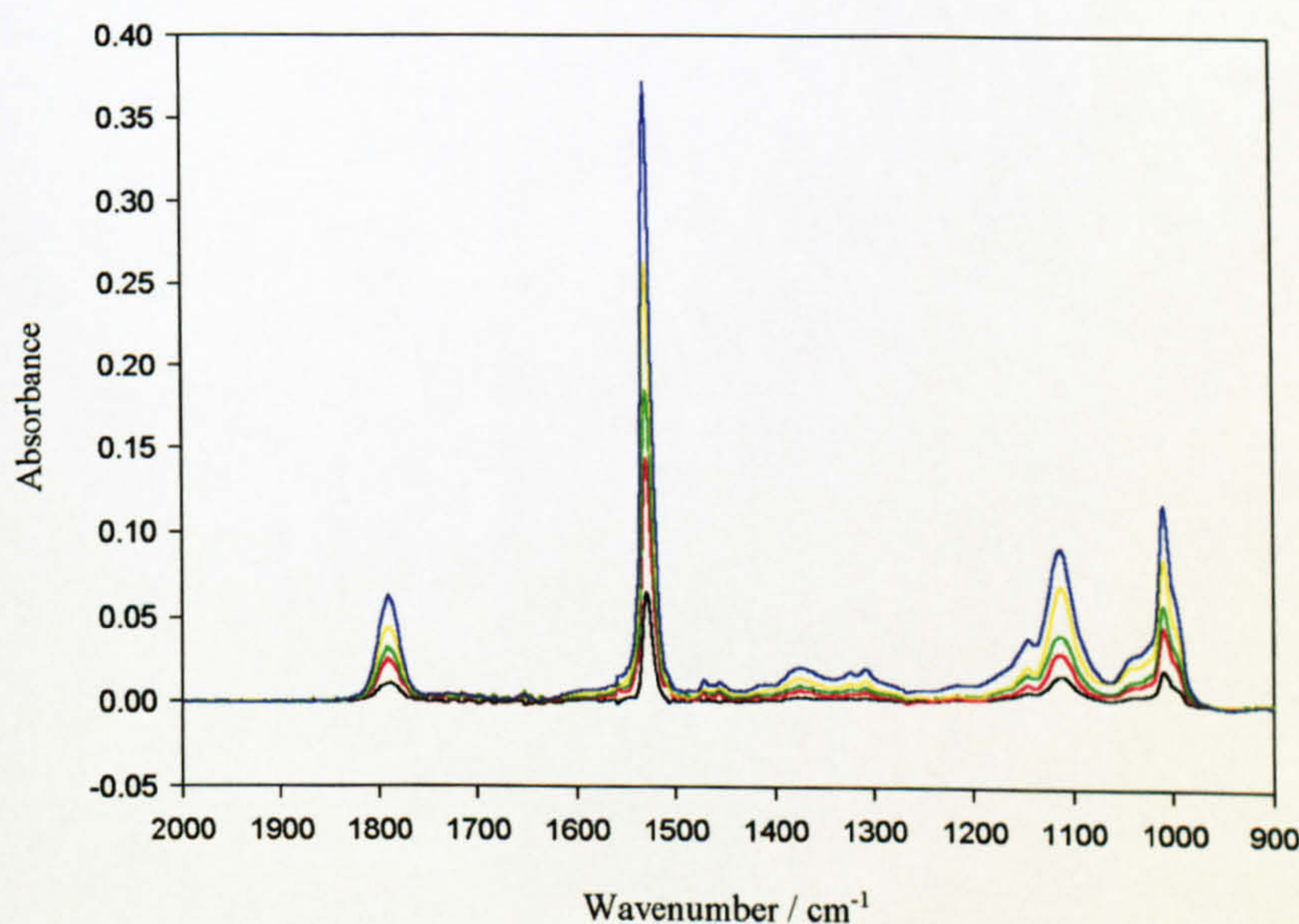


**Figure 3.11:** RAIRS absorbance spectra of unreacted homopolymer of PFP prepared by electrochemical polymerisation and scanning the potential to 1.02 V using a solution containing 20 mM PFP, 0.1 M TEAP and MeCN. Note the peaks at  $1790\text{ cm}^{-1}$  and  $1517\text{ cm}^{-1}$  corresponding to ester stretch and aryl ring stretch respectively.



The spectra collected for homopolymer of PFP show distinct signals corresponding to the activated ester and pentafluorophenyl groups of the PFP species. These are shown in Figure 3.11 where the activated ester can be identified as the C=O stretch can be seen at  $1790\text{ cm}^{-1}$  and aryl ring of the pentafluorophenyl leaving group at  $1517\text{ cm}^{-1}$ .

Experiments were performed to compare the peak heights detected using IR analysis as the number of potential cycles (using cyclic voltammetry) increase during the polymerisation of homopolymer of PFP (Figure 3.12). As the number of potential cycles increase, the peak height increases, which is due to the fact that the polymer films are getting thicker. If quantitative analysis had been necessary, it could have been possible to establish the molar ratios of each polymer film by depositing a series of homopolymers on quartz crystal microbalance crystals and collecting reference spectra of these film using RAIRS.



**Figure 3.12:** RAIR spectroscopy absorbance spectra of unreacted homopolymer films grown to different thickness using cyclic voltammetry. The polymer films were prepared by scanning the potential to 1.02 V in a solution containing 20 mM PFP, 0.1 M TEAP and MeCN. Electrode area =  $1\text{ cm}^2$  and scan rate was  $20\text{ mVs}^{-1}$ . To obtain different thickness each film was grown using a different number of cycles.

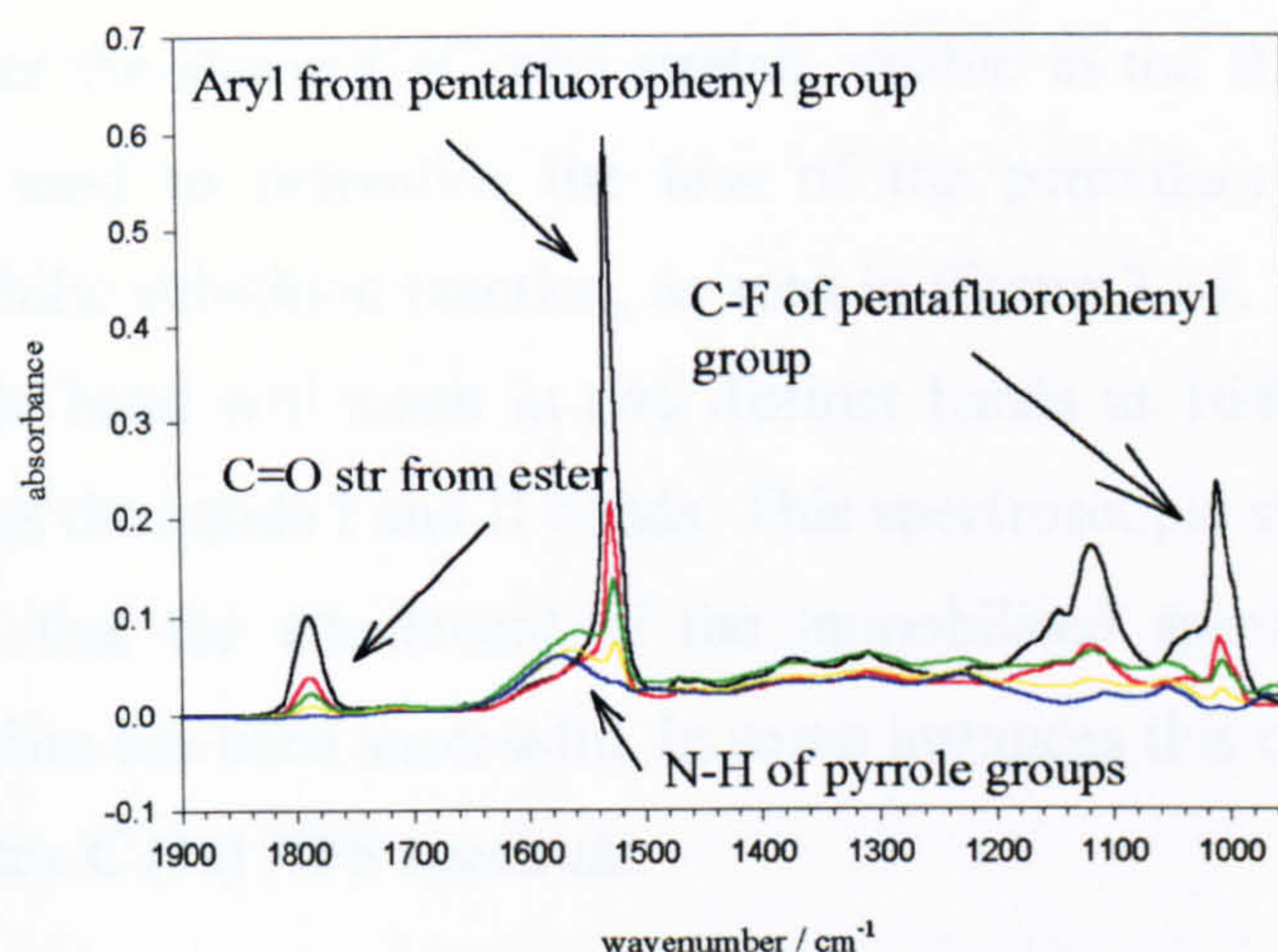


### **3.3.5 Application of RAIR Spectroscopy to Copolymer Analysis**

Using RAIRS measurements, distinct signals corresponding to the activated ester and the pentafluorophenol groups of the PFP species, together with the N-H stretch of the unfunctionalised pyrrole ring were used to demonstrate that a copolymer film has been electrodeposited, Figure 3.13. This series of spectra show that there is a decrease in the ratio of ester stretch band to the N-H band as the amount of PFP within the copolymer film decreases. In addition the aryl ring stretch from the pentafluorophenyl leaving group also decreases as the incorporation of pyrrole increases.

The difficulty in determining the molar coefficients for these vibrations hampers the quantification of the amount of each monomer within the copolymer films using this data and thus the XPS spectra described in section 3.4.2 were used for quantification purposes.





**Figure 3.13:** RAIRS absorbance spectra of (a) homopolymer of PFP and Py/PFP copolymer polymerised by scanning the potential in deposition solution containing 100:0 (black), 95:5 (red), 85:15 (green), 75:25 (yellow), 50:50 (blue) PFP:Py monomer ratio (0.1 M TEAP and MeCN). Electrode area= 1 cm<sup>2</sup> and using a scan rate of 20 mVs<sup>-1</sup>.

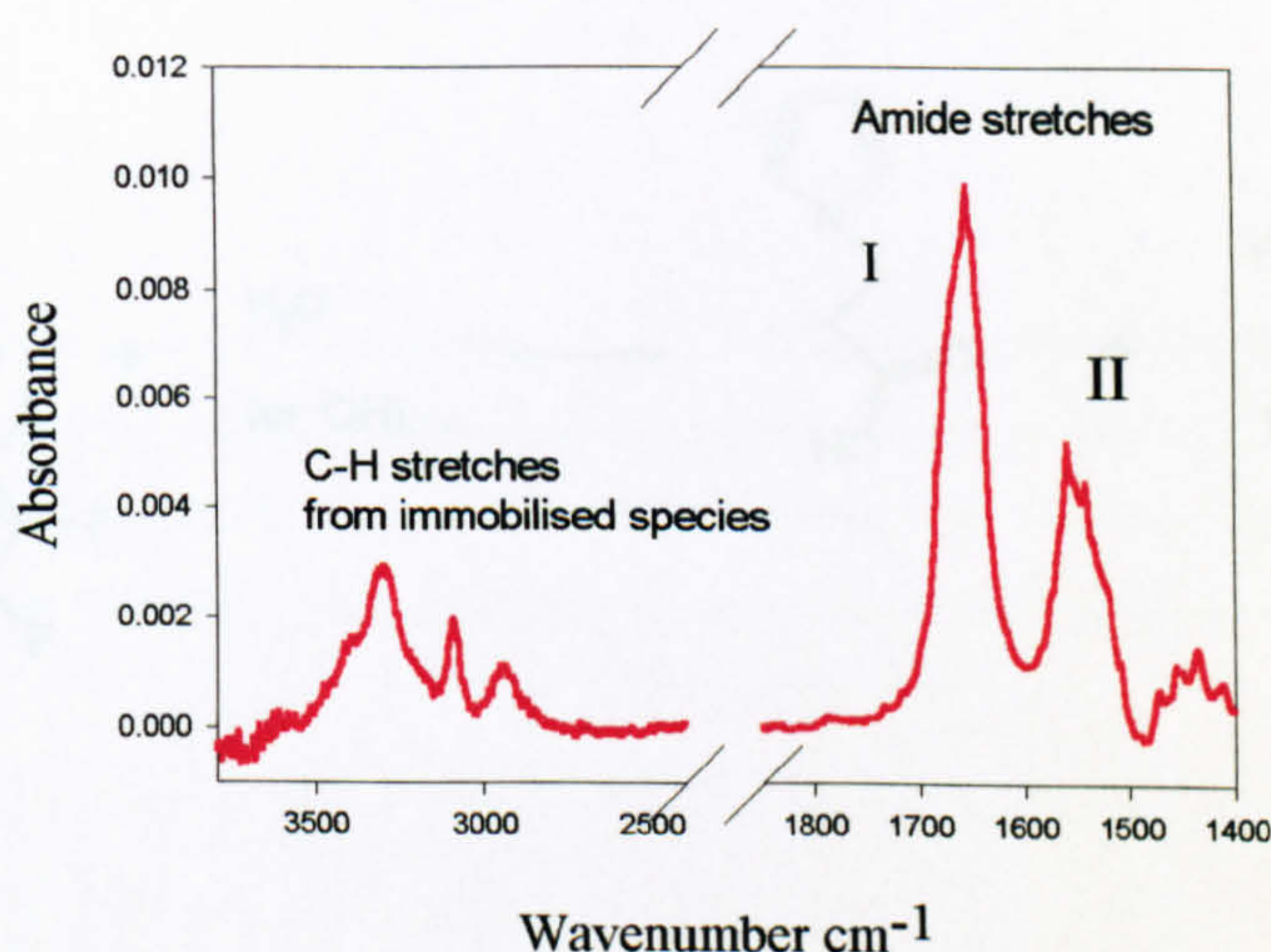
### 3.3.6 Application of RAIR Spectroscopy for Analysis of Polymer Reactions

RAIRS was also used to analyse modified polymeric films after a given immobilisation reaction. If the desired nucleophilic reaction of the amine with the ester groups present within the PFP homopolymer and PFP/Py copolymer films is successful then amide bond formation will occur.

IR analysis presents an ideal method to detect the presence of amide bonds within the polymer matrix. As previously mentioned this technique looks at the bulk composition of the polymer before and after reaction with the desired species. The amide bonds formed during immobilisation enables the progress of the coupling reaction throughout the bulk film to be investigated. This can be easily detected



using reflectance RAIRS spectroscopy to measure the decrease carbonyl vibrational stretch at  $1790\text{ cm}^{-1}$  due to the subsequent removal of the PFP leaving group. Moreover the strong C=C aryl stretch, visible in the RAIRS spectrum at  $1517\text{ cm}^{-1}$  can be used to determine the loss of the pentafluorophenyl group following a nucleophilic substitution reaction, as seen in Figure 3.14. In addition, the formation of an amide bond will result in two distinct bands at  $1640$  and  $1560\text{ cm}^{-1}$ , which are known as the amide I and II bands. This spectroscopic signature makes it possible to confirm that the attachment of the immobilised species via the desired reaction mechanism has been successful. In some instances this can be easier to interpret than a complex C (1s) XPS spectrum.



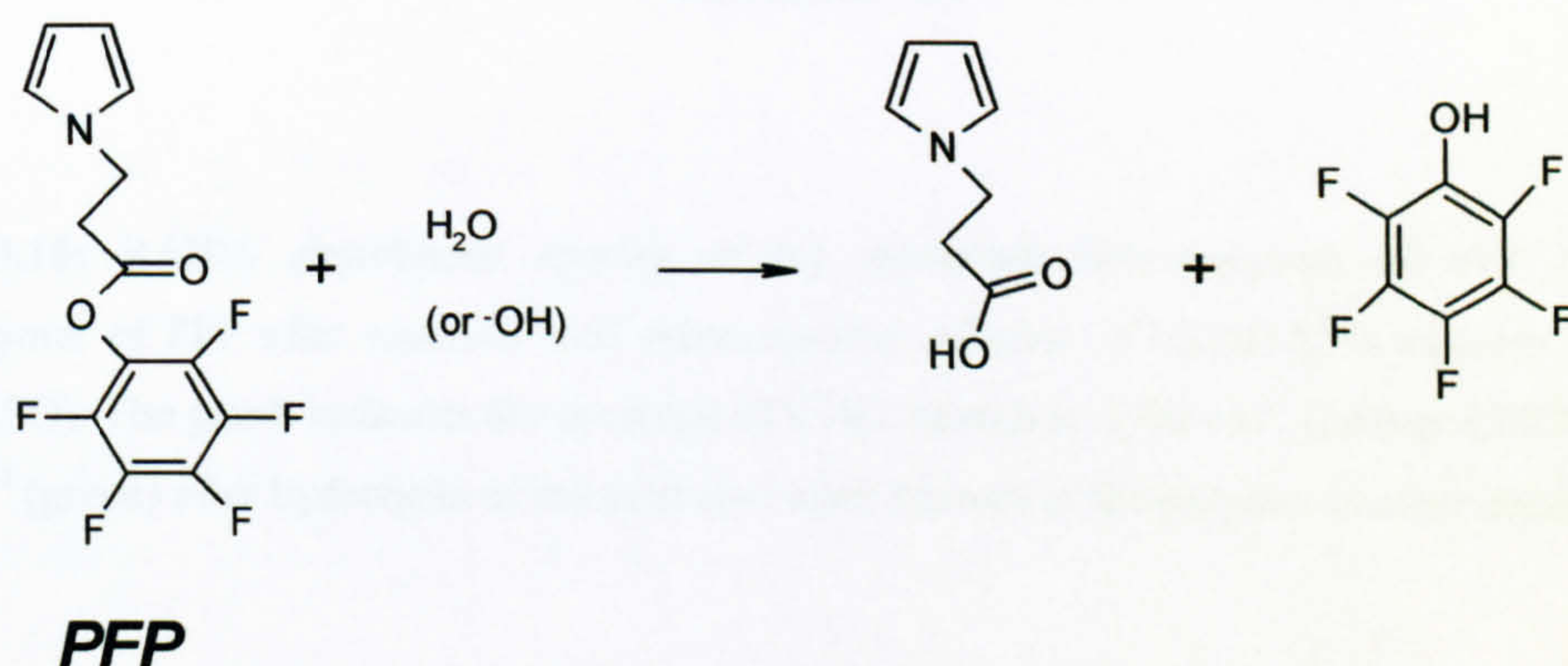
**Figure 3.14:** RAIRS spectrum of PFP homopolymer after substitution reaction with amine species, which leads to the formation of amide I and II bands.

It should be noted that as a consequence of using a pyrrole derivative containing an activated ester group the polymer could be susceptible to irreversible reaction with water molecules or hydroxyl ions causing hydrolysis to form the parent (Pyrrol-1-yl) propanoic acid (see reaction scheme in Figure 3.15).



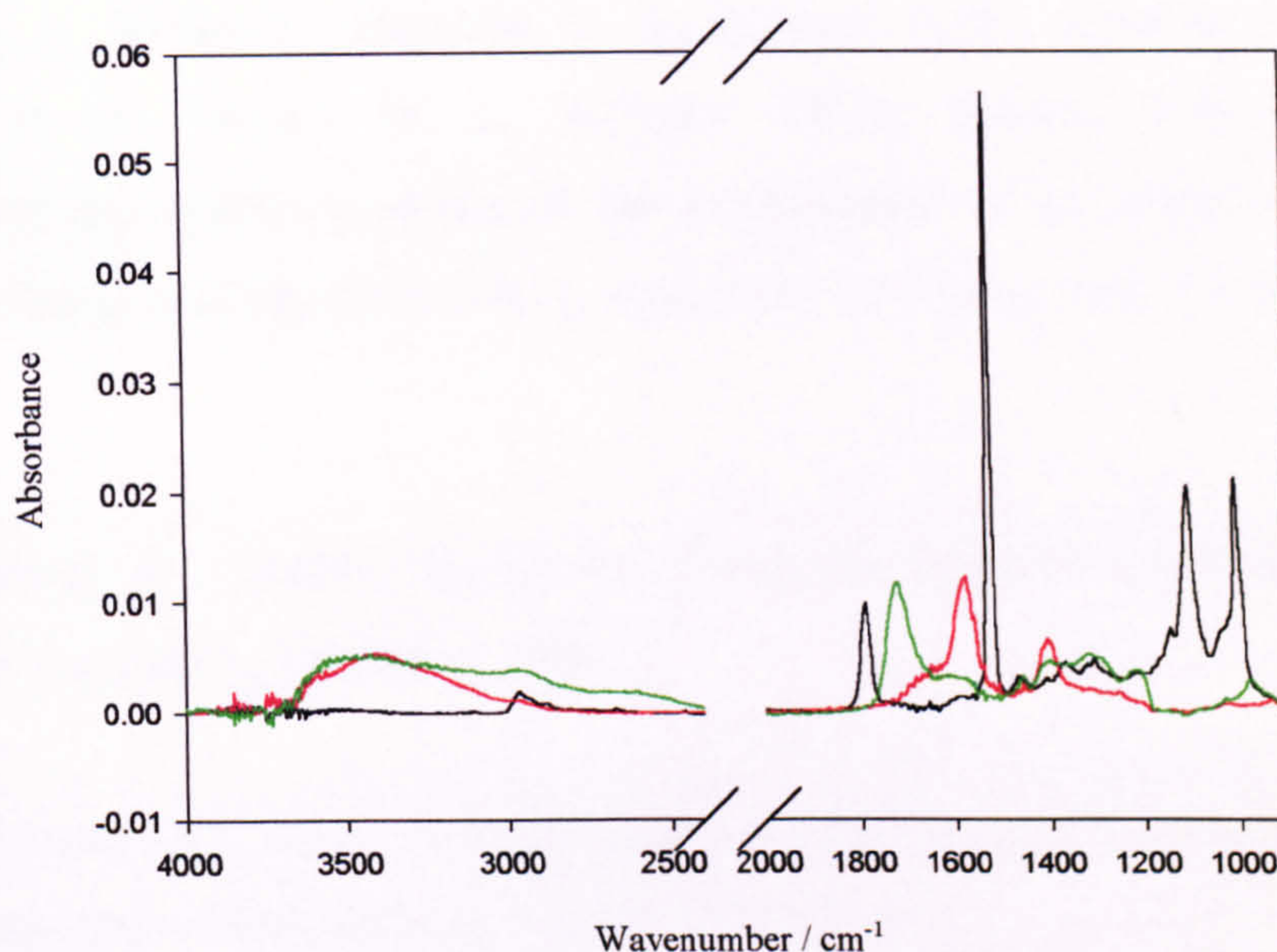
This hydrolysis reaction could be a consequence of the water molecules being present in the amine containing solvent and in the atmosphere. This unwanted side reaction can be stopped or kept to a minimum by using dried solvents and by carefully storing the polymeric films in vacuum desiccator.

The occurrence of this hydrolysis can be determined using both XPS and RAIRS measurements. For example Figure 3.16 shows the RAIRS spectrum of PFP after hydrolysis reaction. Washing the sample in NaOH solution (0.1M) provides confirmation that the band at  $1720\text{ cm}^{-1}$  corresponds to a  $\text{CO}_2\text{H}$  group. The spectra of the washed film shows that the  $1720\text{ cm}^{-1}$  peak has moved to  $1590\text{ cm}^{-1}$ , characteristic of the transform of a  $\text{CO}_2\text{H}$  group to  $\text{CO}_2^-$ . This phenomenon was further investigated in Section 4.2.1.1.



**Figure 3.15:** Reaction scheme for the hydrolysis of PFP. This leads to the production of Py(COOH) and pentafluorophenol.





**Figure 3.16:** RAIRS absorbance spectra of (a) unreacted homopolymer of PFP (black) (b) homopolymer of PFP after reaction with semi-aqueous solution of NaOH (5% aqueous NaOH and 95% DMSO). The graph indicates the presence of COO<sup>-</sup> stretch at 1590 cm<sup>-1</sup> (red) or COOH stretch at 1720 cm<sup>-1</sup> (green) after hydrolysis of the activated ester present in the polymer has occurred.

### 3.4 Summary

Both XPS and RAIRS were utilised to analyse the polymer films before and after modifications were performed. XPS gives information on the interface of each reacted polymer, indicating any changes in polymer composition. RAIRS was mainly used to ensure the desired aminolysis reaction has occurred and also to establish if there was any unwanted side reactions (e.g. hydrolysis).

In addition, it has been illustrated that spectral decomposition of C (1s) XPS signals from electrodeposited polymer interfaces can be used to determine the composition of various Py/PFP copolymer films.



### 3.5 References

1. Glidle, A., Bailey, L., Hadyoon, C. S., Hillman, A. R., Jackson, A., Ryder, K. S., Saville, P. M., Swann, M. J., Webster, J.R.P., Wilson, R.W., Cooper, J.M., "Temporal and spatial profiling of the modification of an electroactive polymeric interface using neutron reflectivity", *Analytical Chemistry*, vol. 73, no. 22, pp. 5596, 2001.
2. Wilson, R. W., Neutron reflectivity of polymer modified electrodes, PhD Thesis, Leicester University, Leicester, 1999.
3. Vickerman, J. C., et al., "Surface Analysis: The Principal Techniques". 1st edition, Chichester: John Wiley & Sons Ltd. pp. 703, 1997.
4. Willard, H. H., "Chemical Analysis of Surfaces", in "Instrumental Methods of Analysis", Van Nostrand: London. pp. 379-402, 1974.
5. Briggs, D., et al., "Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy", 1st edition, Chichester: John Wiley & Sons Ltd., pp. 1-527, 1983.
6. Beamson, G., Briggs, D., "High Resolution XPS of Organic Polymers; The Scienta ESCA300 Database", John Wiley & Sons Ltd., Chichester, 1992.
7. Smith, D. P. E., Horber, J. K. H., Binning, G., Nejoh, N., "Structure, Registry and Imaging Mechanism of Alkylcyanobiphenyl molecules by tunneling microscopy", *Nature*, vol. 344, pp. 641, 1990.
8. Hara, M., Iwakabe, Y., Tochigi, K., Sasabe, H., Garito, A. F., Yamada, A., "Anchoring Structure of Smectic Liquid Crystal Layers on MOS<sub>2</sub> Observed by Scanning Tunneling Microscopy", *Nature*, vol. 344, pp. 228, 1990.



9. Pan, J., Tao, N., Lindsay, S. M., "An atomic force microscopy study of a self-assembled octadecyl mercapto monolayer adsorbed on gold(III) under potential control", *Langmuir*, vol. 9, pp. 1556, 1993.
10. Stange, T. G., Mathew, R., Evans D.F., Hendrickson, W. A., "Scanning tunnelling microscopy and atomic force microscopy characterization of polystyrene spin-coated onto silicon surfaces", *Langmuir*, vol. 8, pp. 920, 1992.
11. Kemp, W., Organic Spectroscopy, third edition, London: MacMillan Education, 1991.
12. Vickerman, J. C., et al., Surface Analysis: The Principal Techniques. 1st edition, Chichester: John Wiley & Sons Ltd. pp. 280, 1997.
13. Vickerman, J. C., et al., Surface Analysis: The Principal Techniques. 1st edition, Chichester: John Wiley & Sons Ltd. pp. 278, 1997.
14. Williams, H.D. and I. Fleming, "Infrared spectra, in Spectroscopic Methods in Organic Chemistry", McGraw-Hill: London, pp. 29-62, 1980.
15. Schweiger, L.F., Ryder, K. S., D.G. Morris, A. Glidle, J.M. Cooper, "Strategies towards functionalised electronically conducting organic copolymers", *Journal of Material Chemistry*, vol. 10, pp. 107, 2000.
16. Ryder, K.S., Schweiger, L. F., Glidle, A., Cooper, J.M., "Strategies towards functionalised electronically conducting organic copolymers: Part 2. Copolymerisation", *Journal of Material Chemistry*, vol. 10, pp. 1785, 2000.
17. Glidle, A., Swann, M. J., Hadyoon, C. S., Cui, L., Davis, J., Ryder, K. S., Cooper, J. M., "XPS assaying of electrodeposited copolymer composition to optimise sensor materials", *Journal of Electron Spectroscopy and Related Phenomena*, vol. 121, no. 1-3, pp. 131, 2001.



18. Vickerman, J. C., et al., "Surface Analysis: The Principal Techniques". 1<sup>st</sup> edition, Chichester: John Wiley & Sons Ltd. pp. 53, 1997.



# Chapter 4

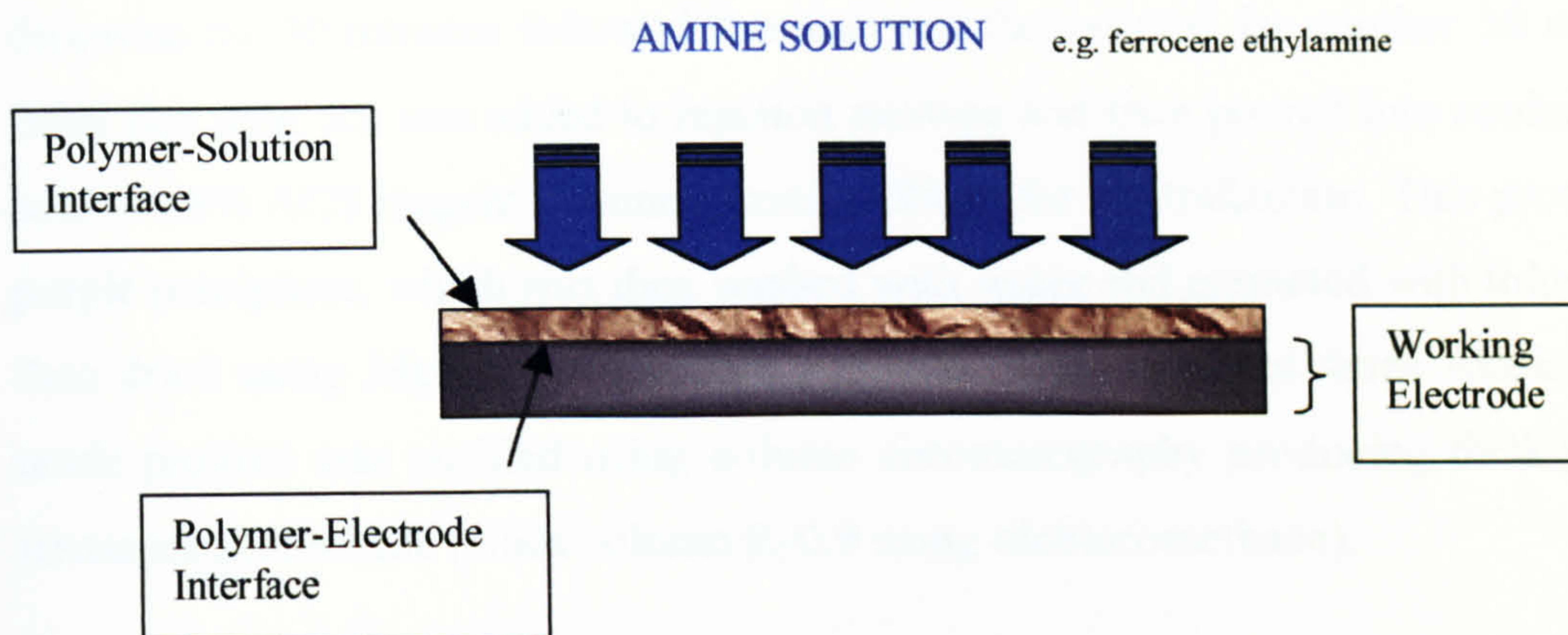
## *Detailed Characterisation of the Reaction Between Poly(PFP) Electrodes and Simple Amines*

### **4.0 Introduction**

This chapter describes both the reaction kinetics together with the influence of polymer composition and solution conditions of the nucleophilic substitution by simple amines with the activated ester groups present in the poly(PFP) system. As previously mentioned, this reaction results in the formation of an amide bond and can be used as the basis of attachment of various reactive species to the polymer film. Two main advantages of using this method of immobilisation are that it has minimal side reactions and produces high reaction yields.

As outlined in Chapter 3, the loss of the fluorine containing pentafluorophenol group is readily detected using RAIRS and XPS methods. Subsequent chapters build on the methods described here and detail the modification of PFP with other motifs used to provide films with functionalities appropriate to biosensing type systems. Thus these measurements provide a ready means to assess the efficacy of a designed modification procedure, particularly aminolysis. The investigations described in this Chapter examine the reaction between poly(PFP) films and the amines, hexylamine and the electrochemical probe, ferrocene ethylamine<sup>1-4</sup>. Ferrocene ethylamine and hexylamine are representative of an electron transfer mediating species and an electro-inactive, non-polar species respectively.





**Figure 4.4:** Schematic diagram showing the reaction of an amine-containing solution with the polymer-modified electrode, illustrating that the first area of contact is the polymer-solution interface.

As seen in Figure 4.4, the first area of contact for the amine containing solution is the polymer-solution interface. Thus it would be expected that the initial reaction is in the interfacial region and the extent of the subsequent modification of the polymer film depends on the rate of transport of the nucleophilic species within the polymer matrix. It is also likely that the porosity and the thickness of the polymer films will determine the rate of the subsequent immobilisation reaction occurring.

## 4.1 Materials

**Solvents:** DMSO (99+%, anhydrous,  $(\text{CH}_3)_2\text{SO}$ ), MeCN (99.5+%, ACS reagent,  $\text{CH}_3\text{CN}$ ), methanol (99.9%, HPLC grade,  $\text{CH}_3\text{OH}$ ), toluene (99.8 %, HPLC grade,  $\text{C}_6\text{H}_5\text{CH}_3$ ), dichloromethane (99.9%, HPLC grade) and diethylether (99+%, ACS reagent) were obtained from Aldrich.

### Synthesis of ferrocene acetonitrile:<sup>5</sup>

50% sodium hydroxide solution (pellets, 97+%, ACS reagent, obtained from Aldrich) was added to (at 0 °C) 2 g of ferrocene aldehyde (cyclopentadienyl(formyl-cyclopentadienyl)iron, 98%, obtained from Aldrich) and 5g nitromethane (96+%, ACS reagent, obtained from Aldrich) in 30 ml of methanol. This was stirred until a



yellow precipitate formed. Adding a further 10ml of methanol and sodium hydroxide dropwise for 30 minutes followed this and was then stirred for another 30 minutes. After this time, ice was added to reaction mixture and then poured into cooled acetic acid (99.8% ACS reagent obtained from Aldrich) for neutralisation. This produced a purple precipitate, which was then washed with water and extracted with toluene and then dried using  $\text{MgSO}_4$  (magnesium sulphate, 97%, obtained from Aldrich). The crude product was purified using column chromatography producing 69% yield of ferrocene acetonitrile (silica column  $R_f$  0.9 using dichloromethane).

The purified product is then used as the starting material for the synthesis of ferrocene ethylamine.

#### **Synthesis of ferrocene ethylamine:<sup>5</sup>**

1 g of Ferrocene acetonitrile was added to a solution of 1 M Lithium aluminium hydride (1.0 M solution in diethyl ether was obtained from Aldrich) and refluxed for 2 hours. After this time the mixture was left to stir overnight at room temperature. The reaction mixture was cooled in ice and 20 ml of water, 15 ml of 20% aqueous sodium hydroxide and 90 ml water was added consecutively. The mixture was then washed with ether and the ethereal layer was then removed by separation. The combined extracts were then saturated with 0.1 M HCl (37% solution in water was obtained from Aldrich). The resulting salt was separated by decantation and was then added to NaOH solution and the mixture was extracted with ether. The ethereal layer was then dried over magnesium sulphate and then evaporated to produce orange oil. This was recrystallised using methanol to produce orange crystals to produce a 70% yield of ferrocene ethylamine.

**Hexylamine and MP-11** were obtained from Sigma

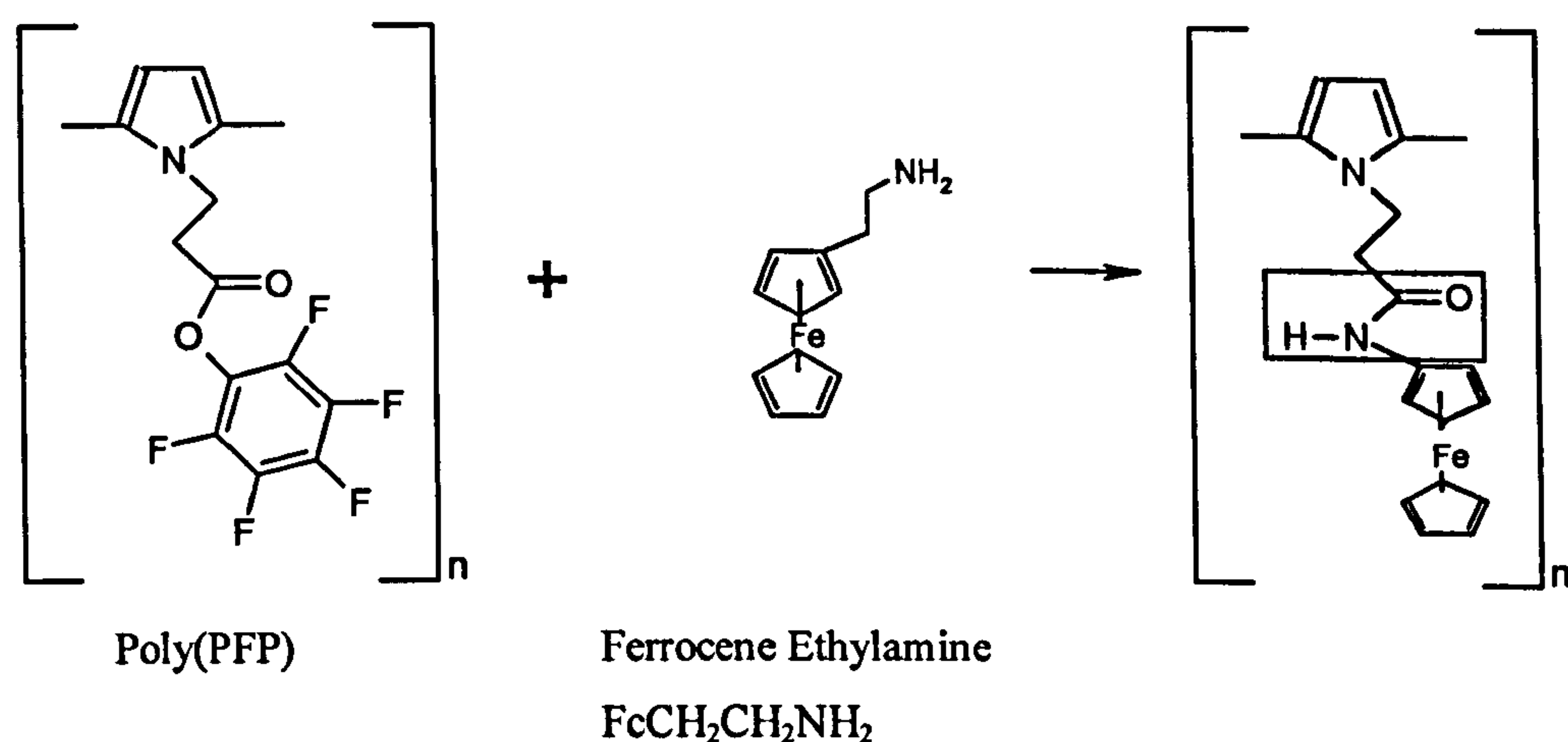
**Interdigitated Electrodes** were prepared using protocol in Chapter 2, Section 2.1.



## 4.2 Results and Discussion

### 4.2.1 Reaction of Polymer with Ferrocene Ethylamine

Preliminary investigations into the coupling reaction of amines with PFP containing polymer films involved the use of ferrocene ethylamine. The reaction scheme of poly(PFP) with  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  is shown in Figure 4.2 and highlighted is the amide bond, which is formed during the nucleophilic reaction. The ferrocene acts as a suitable probe for the nucleophilic coupling reaction with the polymer matrix due to its characteristic one electron electrochemical response and Fe (2p) spectroscopic label for XPS analysis. Whereas as before, XPS measurements quantify the amount of ferrocene on the surface, the magnitude of the current response gives an indication of the quantity of the immobilised species present in the polymer.



**Figure 4.2:** Reaction scheme involving poly(PFP) with redox mediator ferrocene ethylamine. The amine group reacts with the activated ester. Highlighted is the amide bond formed during the immobilisation reaction.

To explore the polymer reaction with ferrocene ethylamine, experiments involving different reaction times and film thickness were performed to investigate the penetration of the amine throughout the polymer film. In addition, ferrocene ethylamine reactions with copolymer films were also carried out to determine



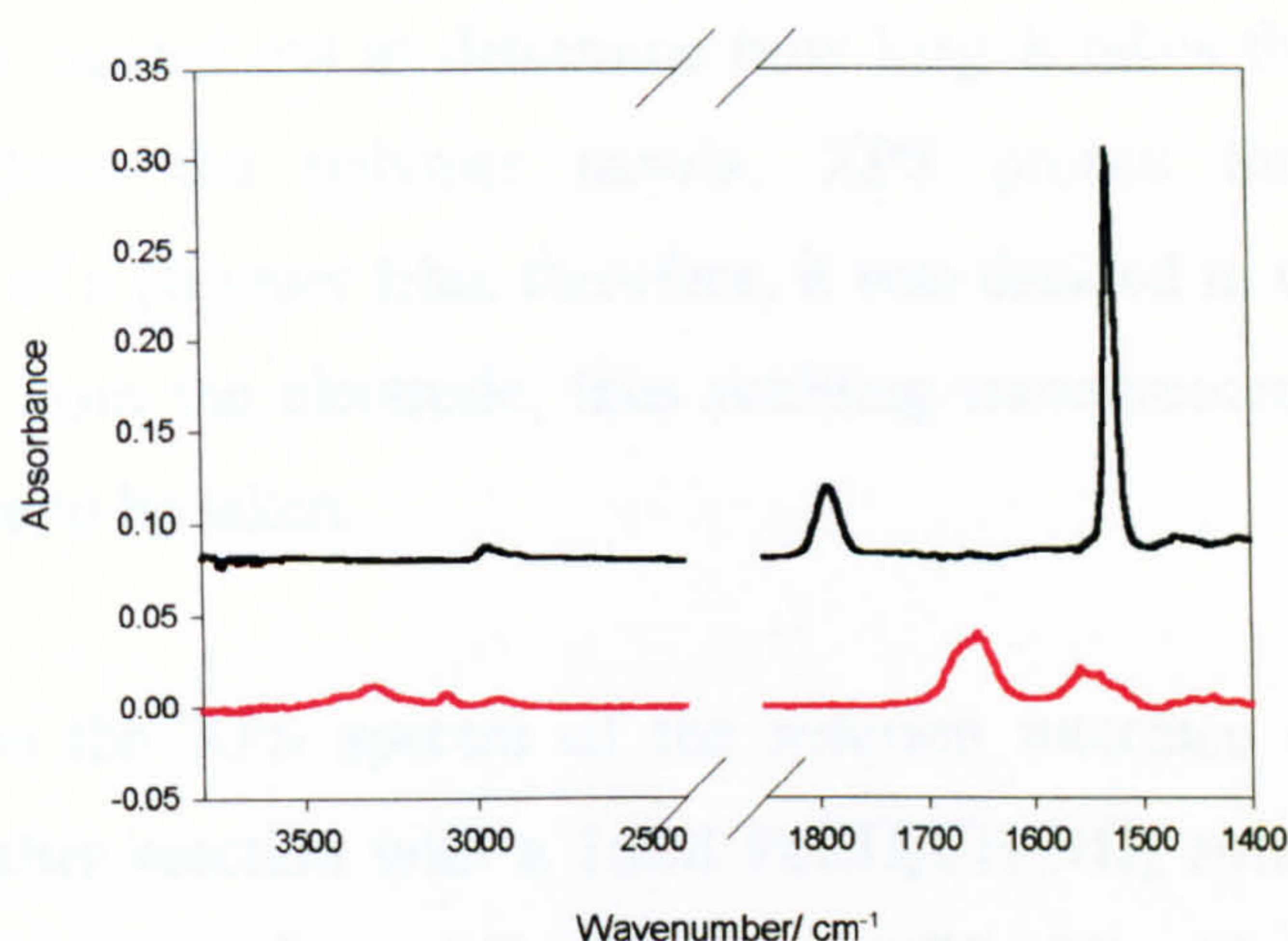
whether a change in polymer composition would lead to change of degree of immobilisation of the redox mediator.

#### **4.2.1.1 Reaction of Poly(PFP) Modified Electrodes with Ferrocene Ethylamine**

As stated in Section 3.4.6, one problem that may be encountered as a consequence of using a pyrrole derivative containing an activated ester group to immobilise amine species, is that the activated ester is susceptible to irreversible hydrolysis. To determine whether aminolysis of the PFP polymer occurs with minimum or no side reaction a film was immersed in a ferrocene ethylamine/DMSO solution until complete reaction has occurred (as indicated by RAIRS). The presence of amide bands in the region between 1500 and 1700  $\text{cm}^{-1}$ , in Figure 4.3, show that the intended reaction is successful. The absence of peaks at 1720  $\text{cm}^{-1}$  ( $-\text{CO}_2\text{H}$ ) and 1590  $\text{cm}^{-1}$  ( $-\text{CO}_2^-$ ) indicate that no hydrolysis (<5%) has occurred.

Investigations of this amine reaction, mainly the time taken for the amine to penetrate throughout the polymer film and the efficiency of the amine reaction are important for optimising protocols used for future studies with biomolecules. Estimations from solution measurements showed that the intrinsic reaction rate between PFP and amine solutions is fast. This was determined by reacting solutions of PFP monomer films with 1 mM amine solutions (ferrocene ethylamine and hexylamine) and determining the extent of reaction using infrared measurements through a thin layer solution cell. RAIRS measurements (as described in Chapter 3) were also used to monitor the extent of reaction of poly(PFP) films.





**Figure 4.3:** RAIRS absorbance spectra of (a) unreacted homopolymer of PFP (black) (b) homopolymer after reaction with 1 mM ferrocene ethylamine solution for 15 minutes (red). Note that the peak at  $1790\text{ cm}^{-1}$  corresponding to the ester group of the unreacted polymer disappears and there is an appearance of distinct peaks around  $1400\text{--}1750\text{ cm}^{-1}$  corresponding to amide groups. Also the presence of C-H stretches around  $3000\text{ cm}^{-1}$  can be seen after reaction with ferrocene ethylamine.



#### 4.2.1.2 General Model for the Reaction of Polymer Film with Solution Species

Experiments were carried out to determine how long it takes the amine species to penetrate throughout the polymer matrix. XPS probes the polymer-solution interfacial region of a polymer film, therefore, it was decided to use adhesive tape to remove the film from the electrode, thus enabling measurements of the polymer-electrode interface to be taken.

Figure 4.5 shows the XPS spectra of the solution interface corresponding to a poly(PFP) film after reaction with a 1mM  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solution for 3 minutes. Clearly there is complete loss of F (1s) signal (687 eV) and the appearance of a strong Fe (2p) doublet signal (between 705 and 720 eV) indicating a complete reaction within this time interval. However, on looking at the polymer-electrode interface it can be seen that only ca 40% (Figure 4.5 (b)) of the polymer film has reacted (approximately 60 % decrease in F (1s) signal and 40 % increase in Fe (2p) signal with respect to the completely reacted homopolymer layer). These results suggest that there is a minimum time requirement for the amine to diffuse through the polymer layer (approximate film thickness of 100 nm). The film thickness was measured using the Dektak surface profiler, this involves the use of a needle to scan the electrode surface and a laser measures the deflections (resolution depends on the surface and is to 10-15 nm). These results also demonstrate that for partially reacted films the extent of reaction is not homogeneous throughout the film thickness. In addition RAIRS measurements (Figure 4.8) can be used to estimate the time taken for penetration and complete reaction throughout the film as discussed below. As may be expected, the reaction time of the film with the amine is reactant concentration dependent. Solutions containing a high amine concentration react more rapidly than a lower amine concentration. However, since lower concentrations are only obtainable when using some biomolecules, the low concentration studies outlined below mimic reaction conditions that may be used in future experiments.

From examination of the Fe (2p) and F (1s) region in the XPS spectra collected, it is possible to observe that by altering the reaction time between PFP and the nucleophilic species there is a progressive decrease in F (1s) and increase in Fe (2p)

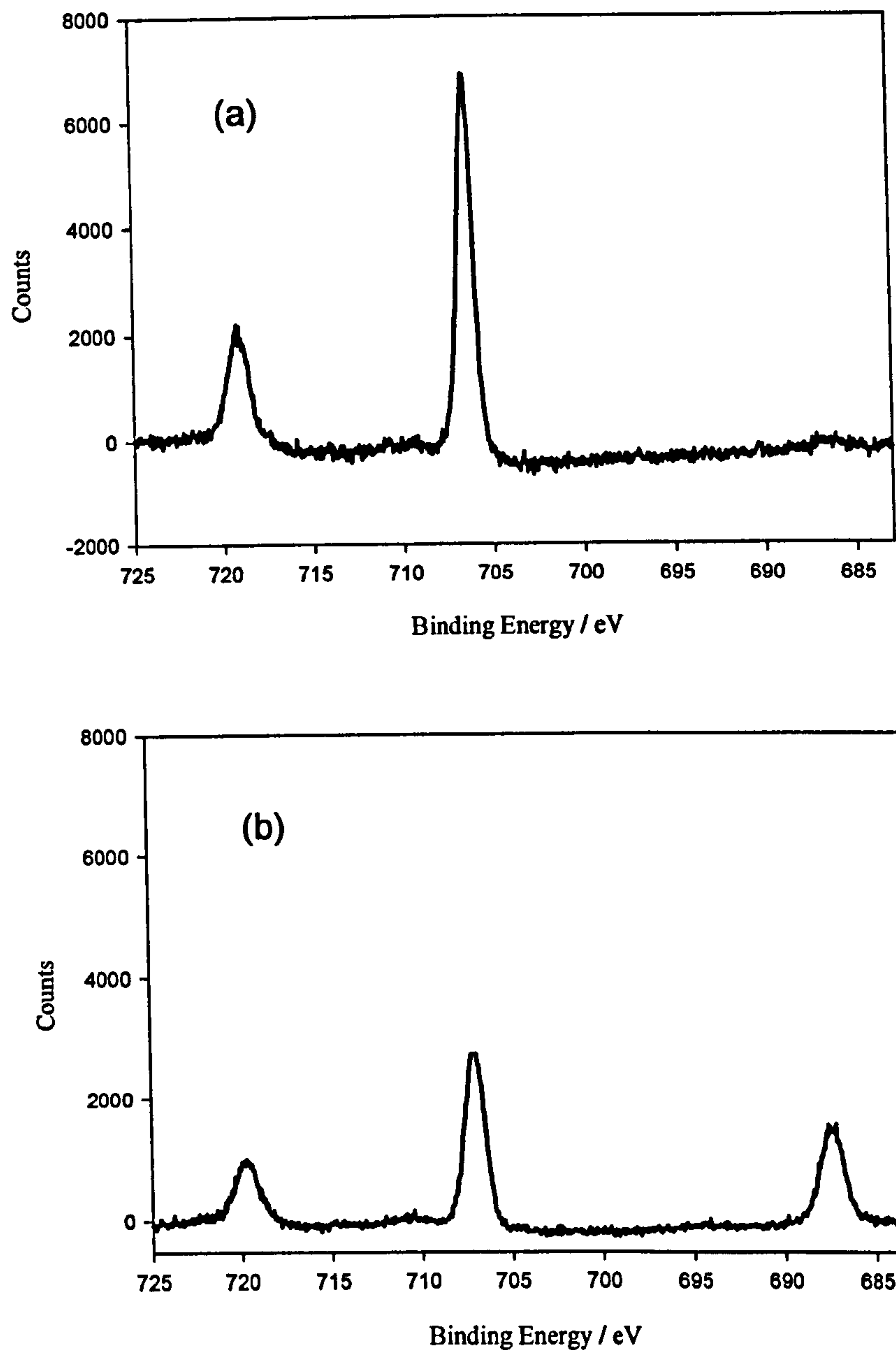


peaks (Figure 4.6). The attractive feature of the XPS technique that is of significance is the ability to identify particular functional groups on the sample being analysed. In this case it can be seen in the C (1s) spectra, in Figure 4.7, that there is a decrease in C-F peak at 287 eV. This is due to the removal of pentafluorophenyl group after substitution with  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  and there is also an increase in C-N group as a result of formation of amide bond.

Figures 4.6 and 4.7 details the time dependant experiments performed to follow the reaction of the homopolymer and  $\text{FcCH}_2\text{CH}_2\text{NH}_2$ . Clearly soaking the polymer coated electrode in the solution for the required time can control the degree of polymer reaction. Although XPS shows Fe present on the film surface (depth of approximately 8 nm), it does not show the degree of immobilisation of the redox species throughout the bulk of the polymer matrix (formation of the amide bond). By using RAIRS (Figure 4.8) it is possible to measure the absorbance of the amide stretches present at 1540 and 1680  $\text{cm}^{-1}$ , corresponds to bonds formed during the course of the reaction.

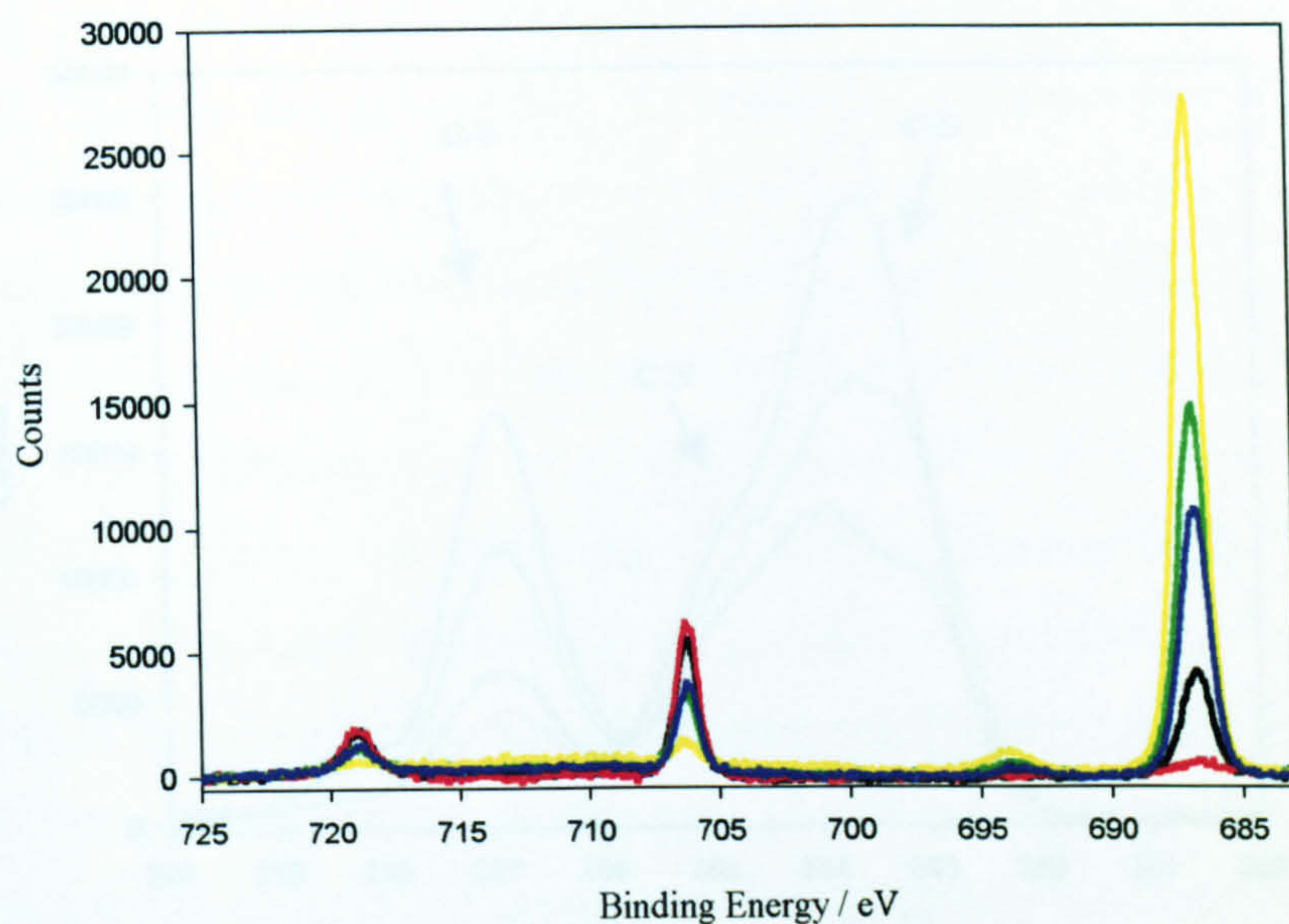
As the reaction time increases there is a progressive decrease in ester stretch at 1790  $\text{cm}^{-1}$ . This change is used in Figure 4.9 to assess the degree of reaction of the polymer with the reactant. Concomitant with this decrease in ester bond stretching, the aryl stretch (approximately 1520  $\text{cm}^{-1}$ ) decreases due to the removal of the pentafluorophenyl leaving group during the nucleophilic reaction. The peak at approximately 1000  $\text{cm}^{-1}$  can be attributed to the C-F stretching vibrations of the pentafluorophenyl groups. Thus, Figure 4.9 shows the % decrease in absorbance at 1790  $\text{cm}^{-1}$  and the measured absorbance at 1670  $\text{cm}^{-1}$  corresponding to the ester stretch and amide bands respectively.





**Figure 4.5:** Fe (2p) and F (1s) XPS spectra (TOA 90 degrees) of (a) the polymer-solution interface of the homopolymer of PFP reacted with ferrocene ethylamine and DMSO solution for 3 minutes (b) the polymer-electrode interface after 3 minute reaction with ferrocene ethylamine and DMSO solution.

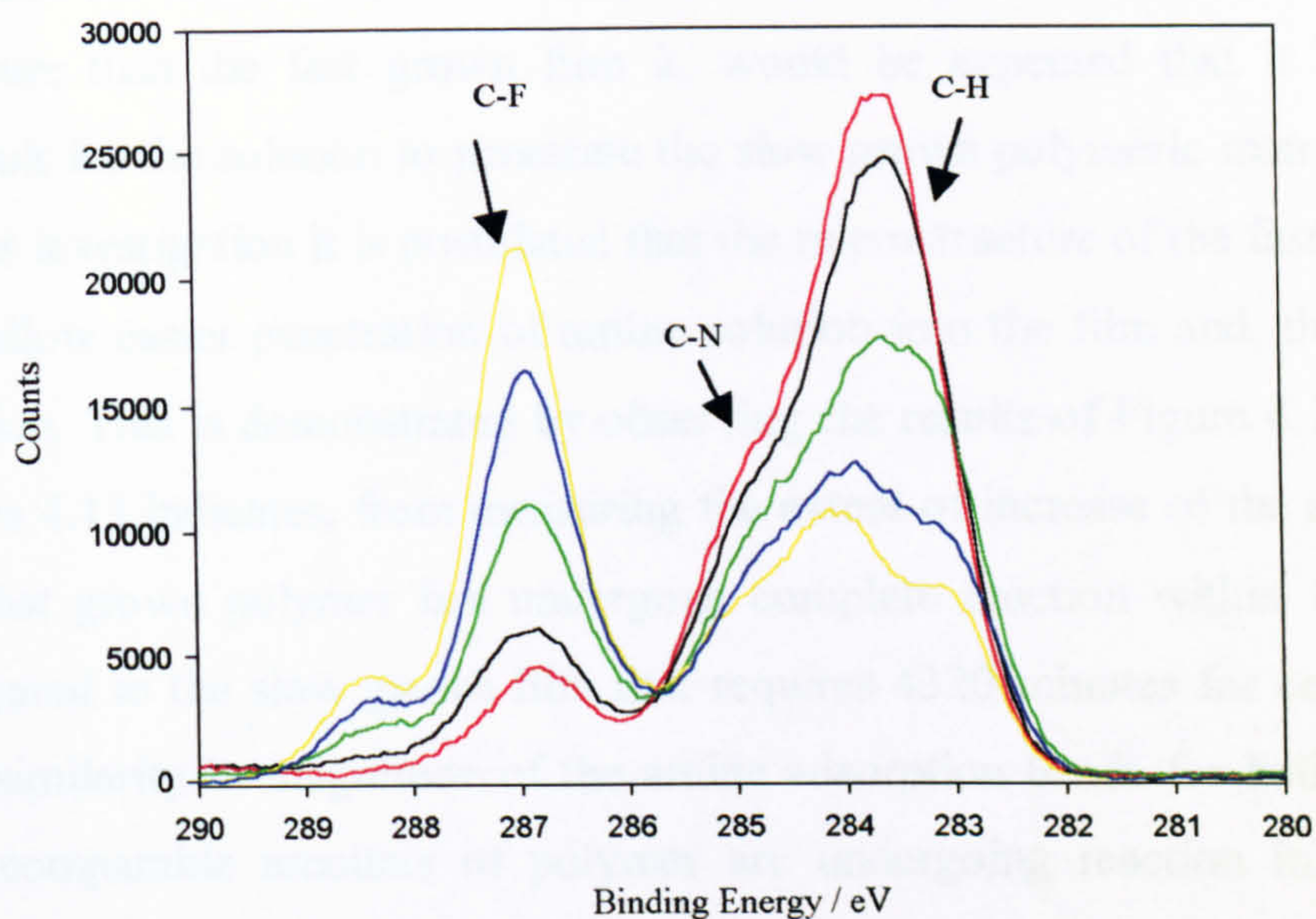




**Figure 4.6:** F (1s) and Fe (2p) XPS spectra (TOA 90 degrees) of homopolymer reacted with ferrocene ethylamine and DMSO solution (0.1 mM) at various time intervals of 30 seconds (yellow), 4 minutes (green), 6 minutes (blue), 8 minutes (black) and 30 minutes (red). There is a decrease in F (1s) signal as Fe (2p) signal increases with time.

Clearly it takes a significant period of time for the reactant to completely penetrate the film and, consequently, for the reaction to occur throughout the film. Therefore, the time necessary for complete reaction of polymeric films of varying thicknesses will increase as the thickness increases. This is illustrated in Figure 4.10 for films of approximate thicknesses between 25 and 100 nm (measured using the Dektak surface profiler). These results are summarised in Figure 4.11 as it shows the percentage decrease of the ester stretch for films of thickness: 25, 50, 75 and 100 nm.





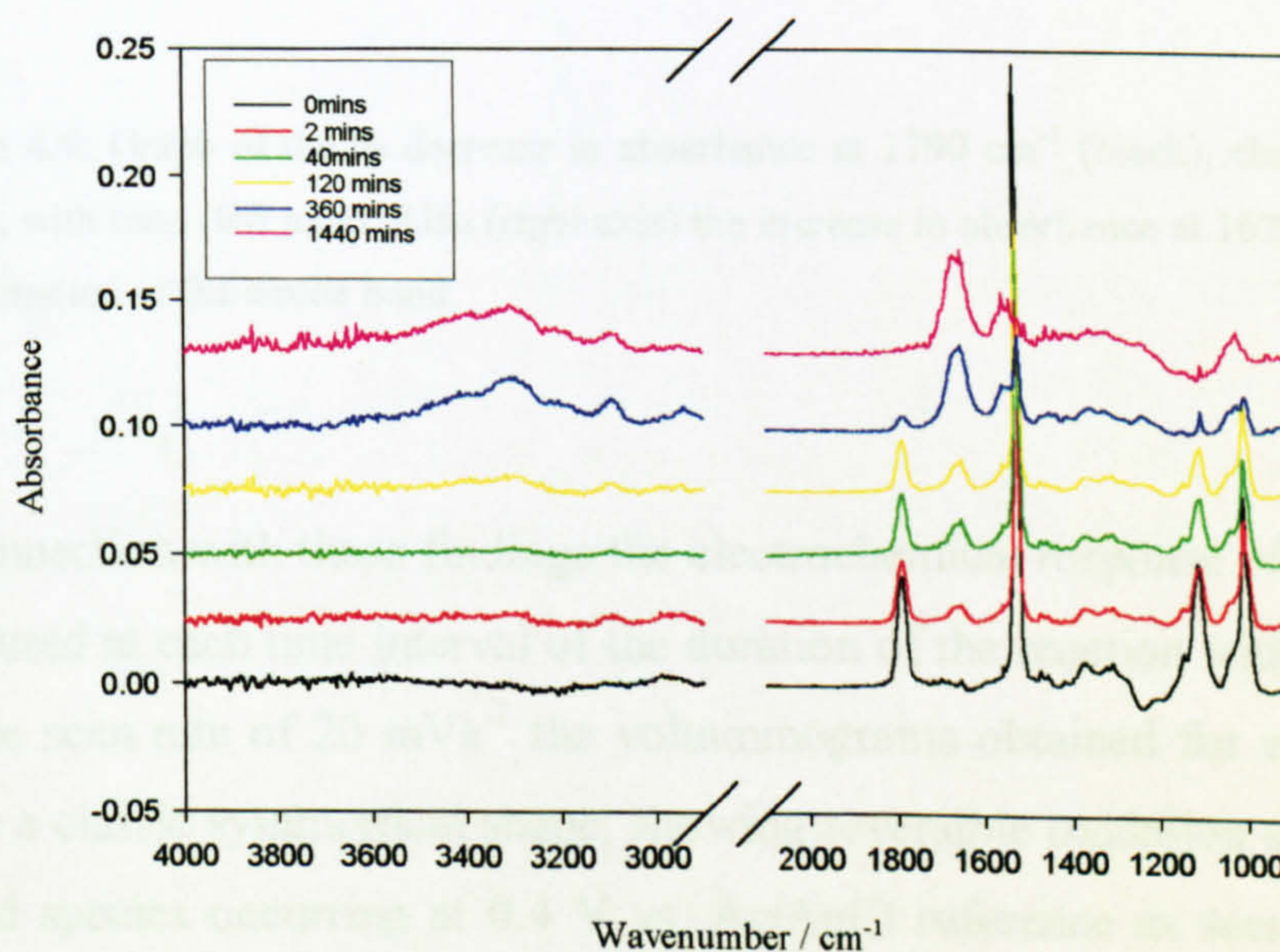
**Figure 4.7:** C (1s) XPS spectra (TOA 90 degrees) of homopolymer of PFP reacted with ferrocene ethylamine and DMSO solution (0.1 mM) at various times during the course of reaction; 30 seconds (yellow), 4 minutes (blue), 6 minutes (green), 8 minutes (black) and 30 minutes (red). Note the decrease in C-F peak at 287 eV and signal at 284 eV, corresponding to C-H groups, increases with time.

Further investigations were carried out examining how the internal microstructural differences of the polymer can affect the rate of reaction of the PFP with the amine solution. The polymers were grown to different potential limits using the same method outlined in Chapter 2 and, therefore, producing fast and slow electrodeposited polymer films. It was anticipated that changing the growth conditions of electrodeposition would cause a change in the polymer microstructure.

RAIRS measurements to monitor the rate of reaction of fast and slow grown films, Figure 4.12, have shown that there was a change in the rate of nucleophilic reaction. These results show that the percentage decrease in ester stretch at  $1790\text{ cm}^{-1}$  for both the fast and slow grown film undergoes an initial rapid decrease when immersed into the solution followed by a slower decrease that is dependent on film growth



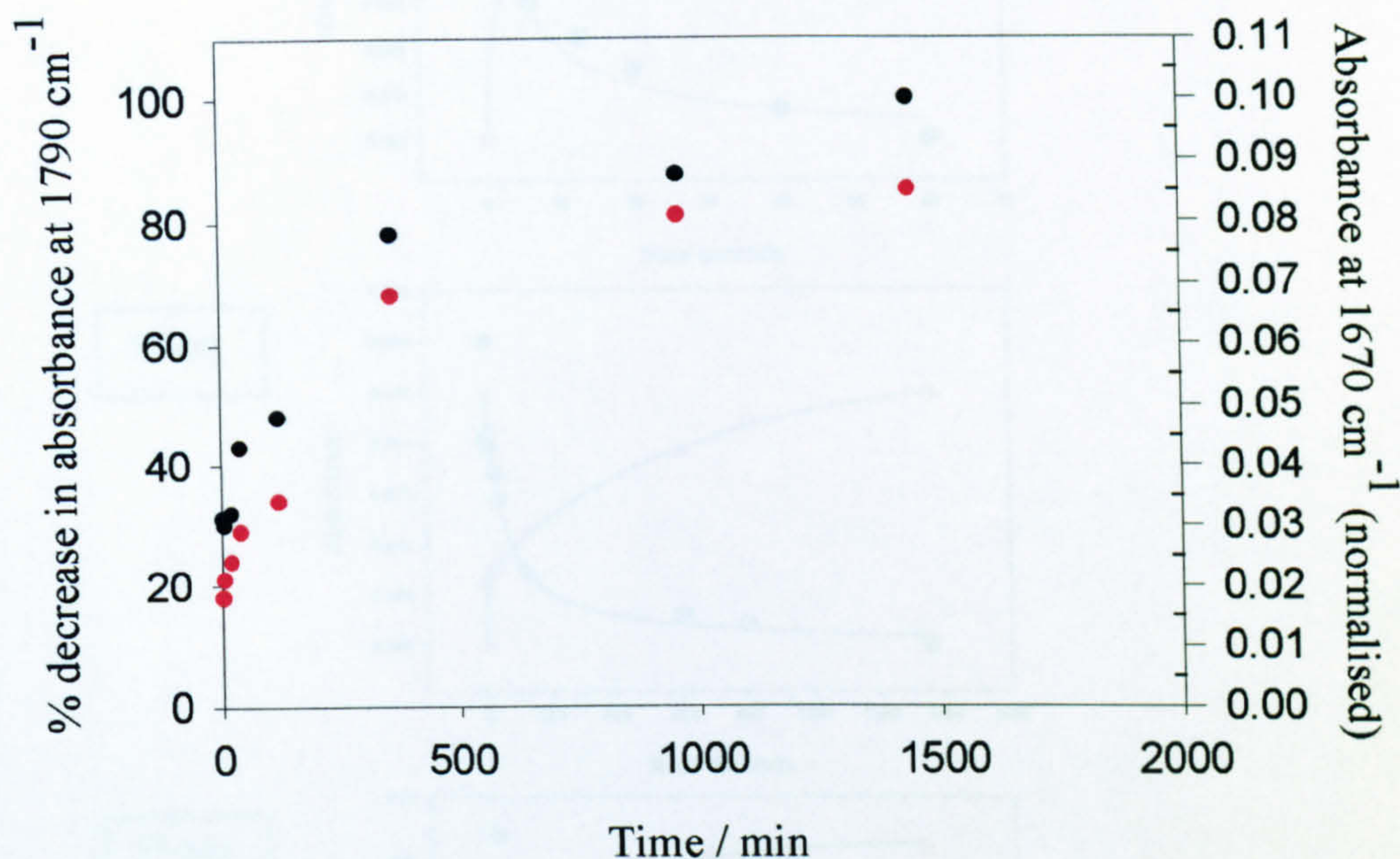
conditions. This may be explained by the fact that when both films are immersed into the solution, diffusion into the top layers will occur at the same rate as the solution will penetrate this region of the matrix with ease. If the underlying structure is different for both films, for example, the slow grown film has a more compact structure than the fast grown film it, would be expected that it would be more difficult for the solution to penetrate the slow grown polymeric matrix. At the outset of this investigation it is postulated that the microstructure of the fast grown polymer will allow easier penetration of amine solution into the film and, therefore, a faster reaction. This is demonstrated by observing the results of Figure 4.12. Furthermore, Figure 4.13 indicates, from measuring the extent of increase of the amide bond, that the fast grown polymer has undergone complete reaction within 1440 minutes as compared to the slow grown film that requires 4320 minutes for complete reaction. The similarity in magnitude of the amide adsorption bands for both films indicates that comparable amounts of polymer are undergoing reaction in both cases and validate reaction time.



**Figure 4.8:** RAIRS absorbance spectra of PFP polymer (film thickness of 50 nm) after reaction with 0.1 mM ferrocene ethylamine and DMSO solution for the following times; 0 minutes (black), 2 minutes (red), 40 minutes (green), 120 minutes (yellow), 360 minutes (blue) and 1440 minutes (pink).



There is a decrease in C-F stretch and increase in amide bond stretches during the course of the reaction.

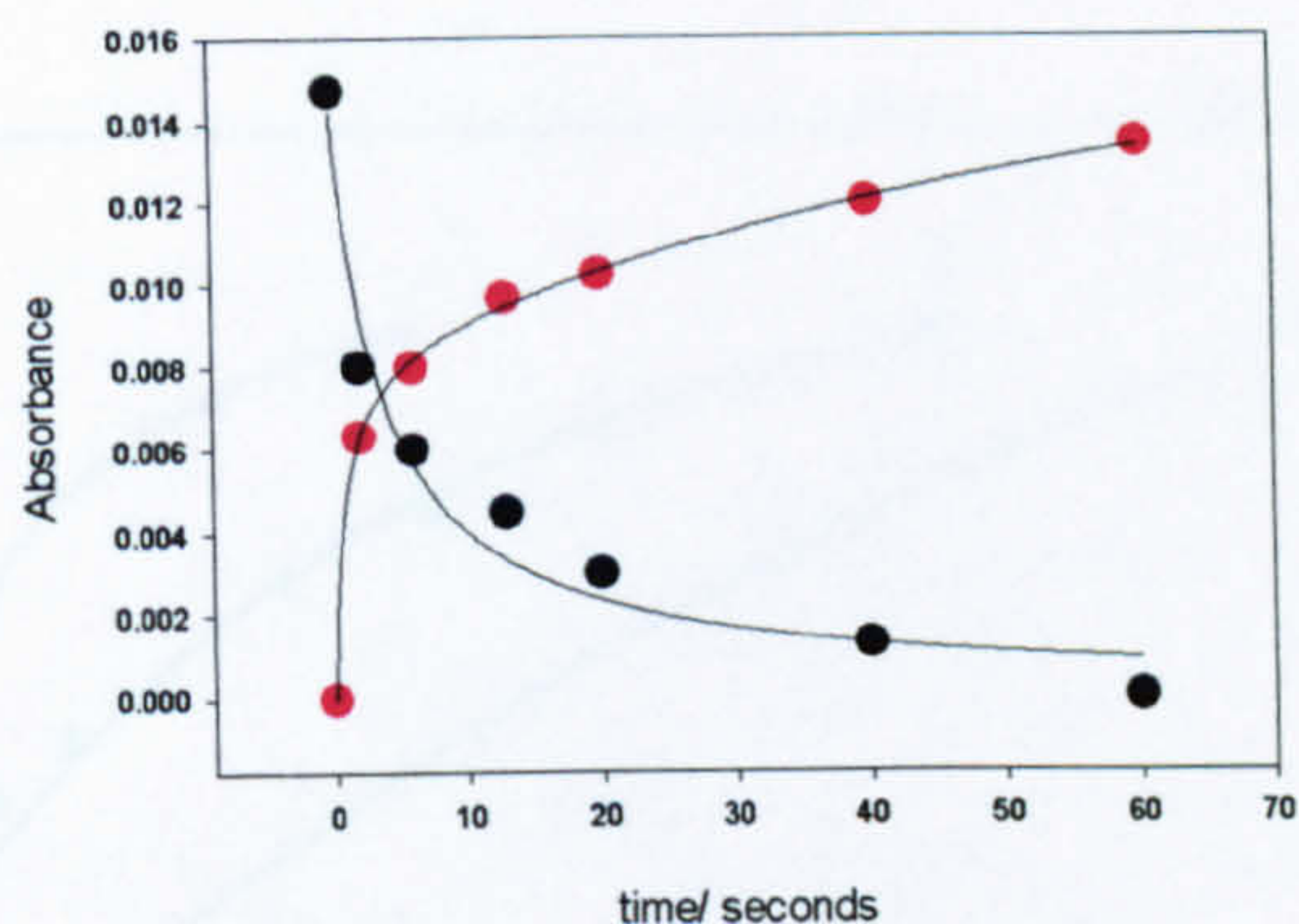


**Figure 4.9:** Graph of the % decrease in absorbance at 1790 cm<sup>-1</sup> (black), characteristic of the ester region, with time (left axis). Also (right axis) the increase in absorbance at 1670 cm<sup>-1</sup> (red), indicating the formation of the amide bond.

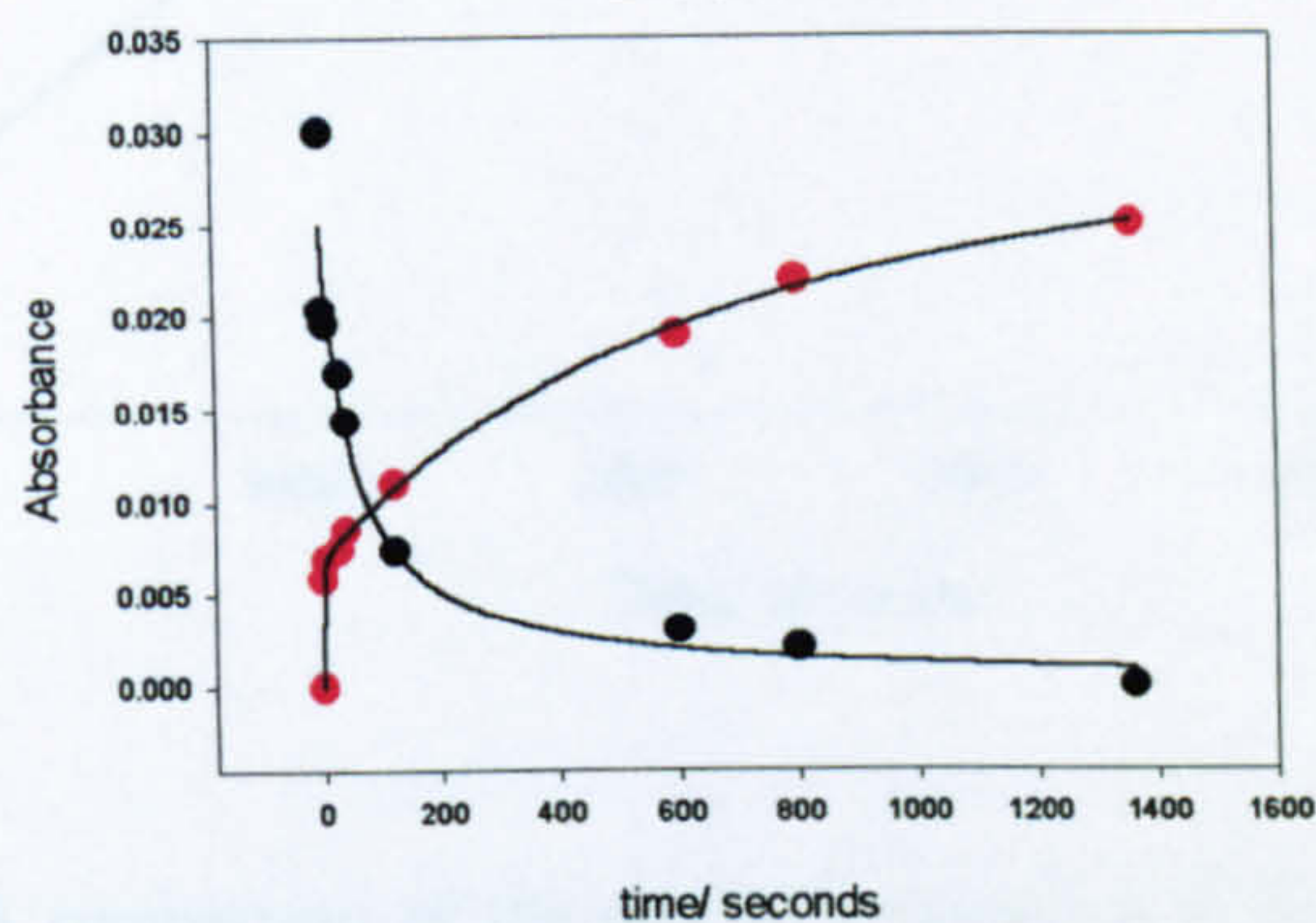
In connection with these findings the electrochemical response of the electrodes was measured at each time interval of the duration of the reaction with the redox species. At the scan rate of 20 mVs<sup>-1</sup> the voltammograms obtained for each of the samples show a classic symmetrical shape, showing reversible oxidation and reduction of the bound species occurring at 0.4 V vs. Ag|AgCl reference as seen in Figure 4.14. It was confirmed that this was reversible reduction system as the peak potential is constant and independent of scan rates as seen in Figure 4.15.



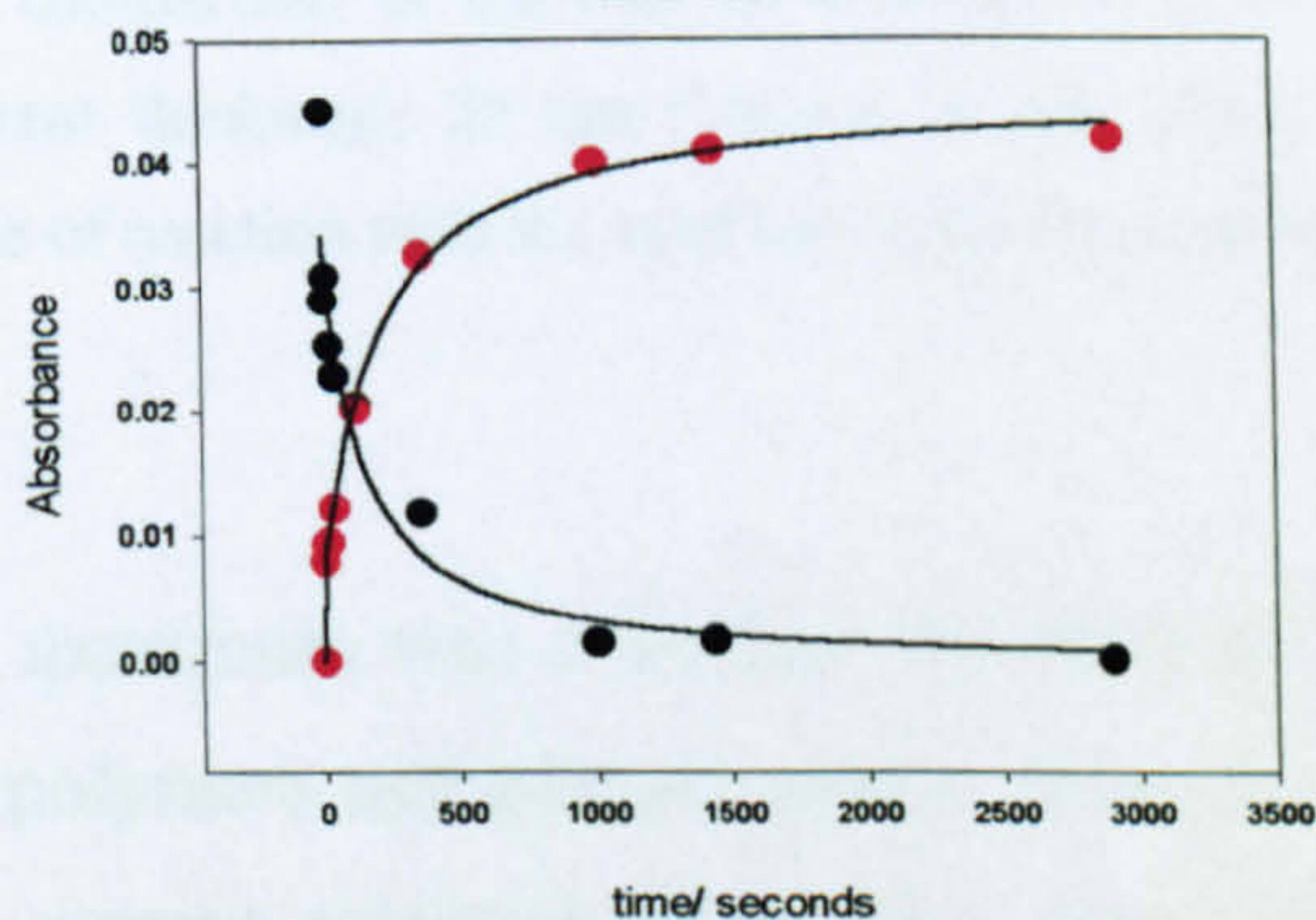
25 nm



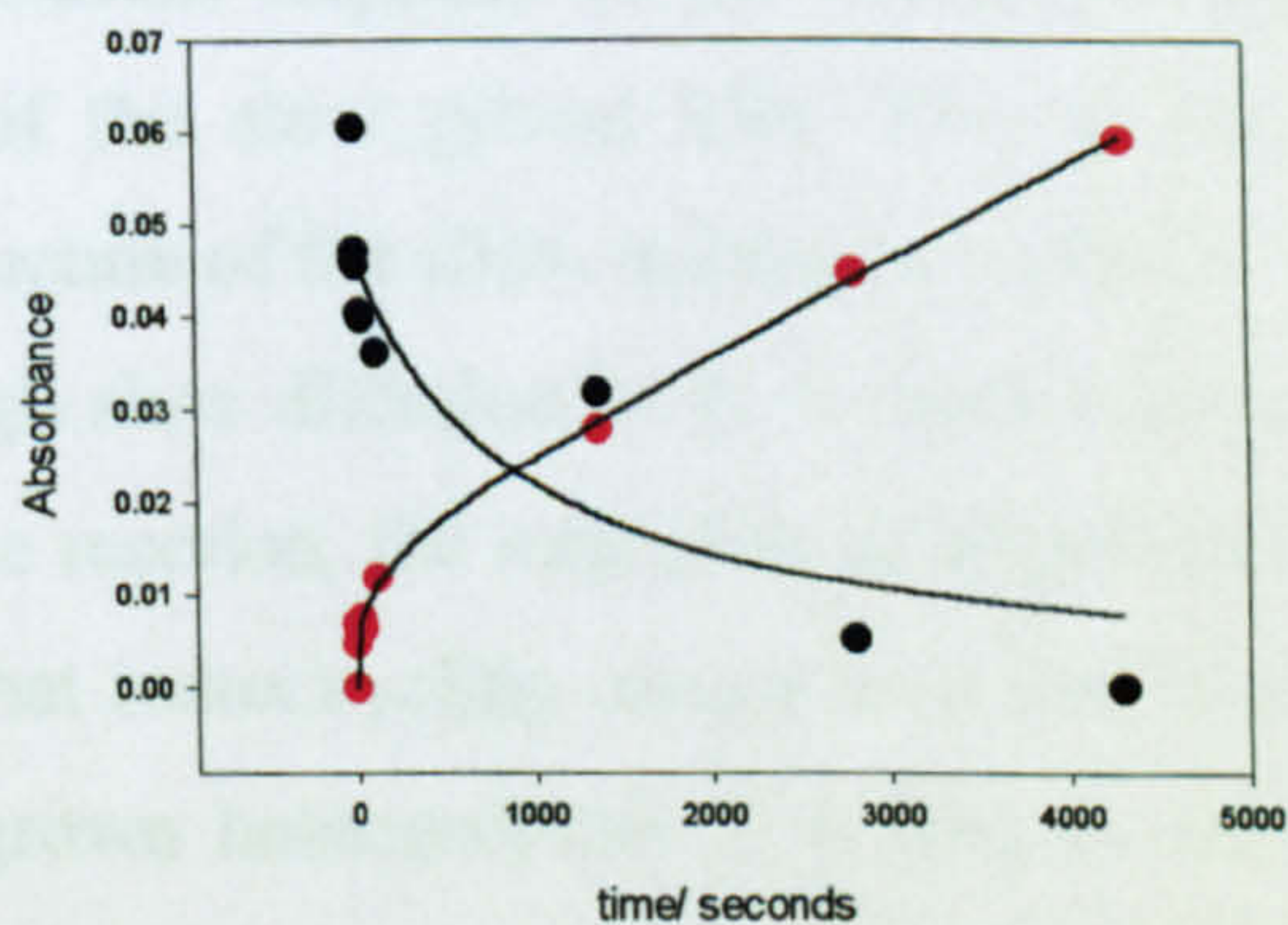
50 nm



75 nm

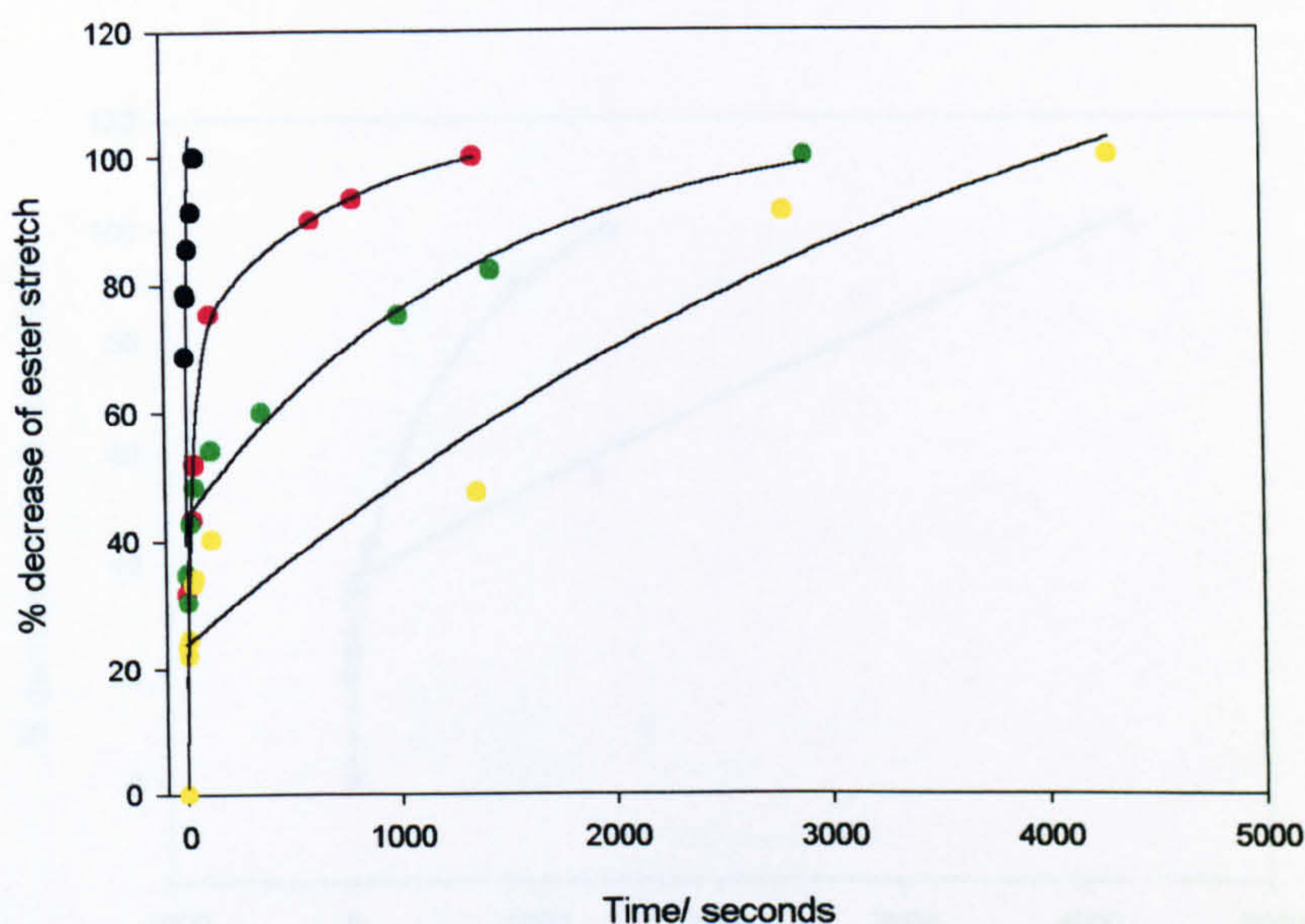


100 nm



**Figure 4.10:** Graph of the decrease in absorbance at  $1790\text{ cm}^{-1}$  (black) (characteristic of the ester region) and the increase in absorbance at  $1670\text{ cm}^{-1}$  (red) (indicating the formation of the amide bond) for poly(PFP) films of different thickness (25, 50, 75 and 100 nm) during reaction with ferrocene ethylamine and DMSO

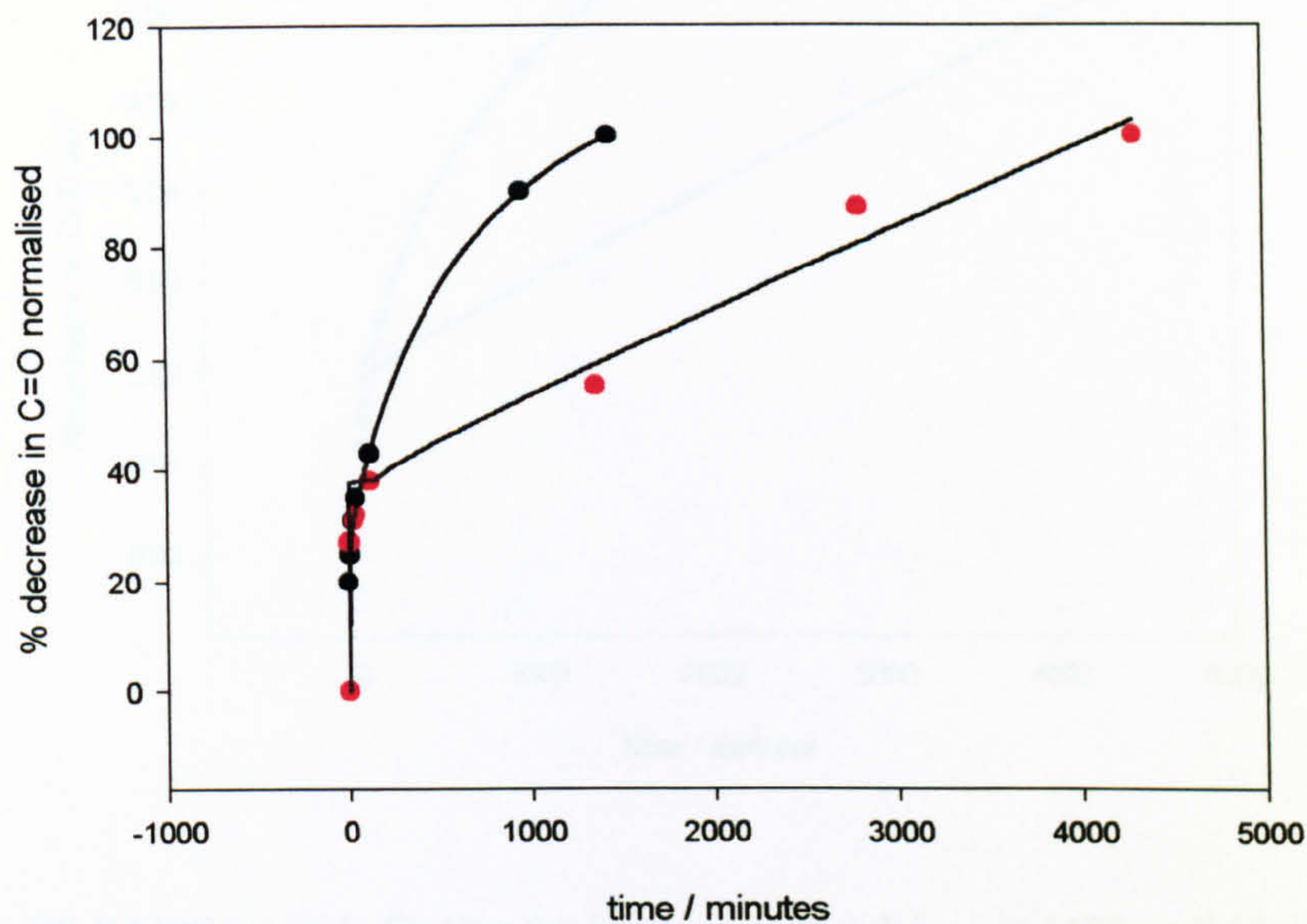




**Figure 4.11:** FT-RAIRS comparison of the rate of decrease in  $\text{C}=\text{O}$  absorbance at  $1790\text{ cm}^{-1}$  of poly(PFP) films of different thickness: 25 nm (black), 50 nm (red), 75 nm (green) and 100 nm (yellow), during the course of reaction with 0.1 mM ferrocene ethylamine and DMSO.

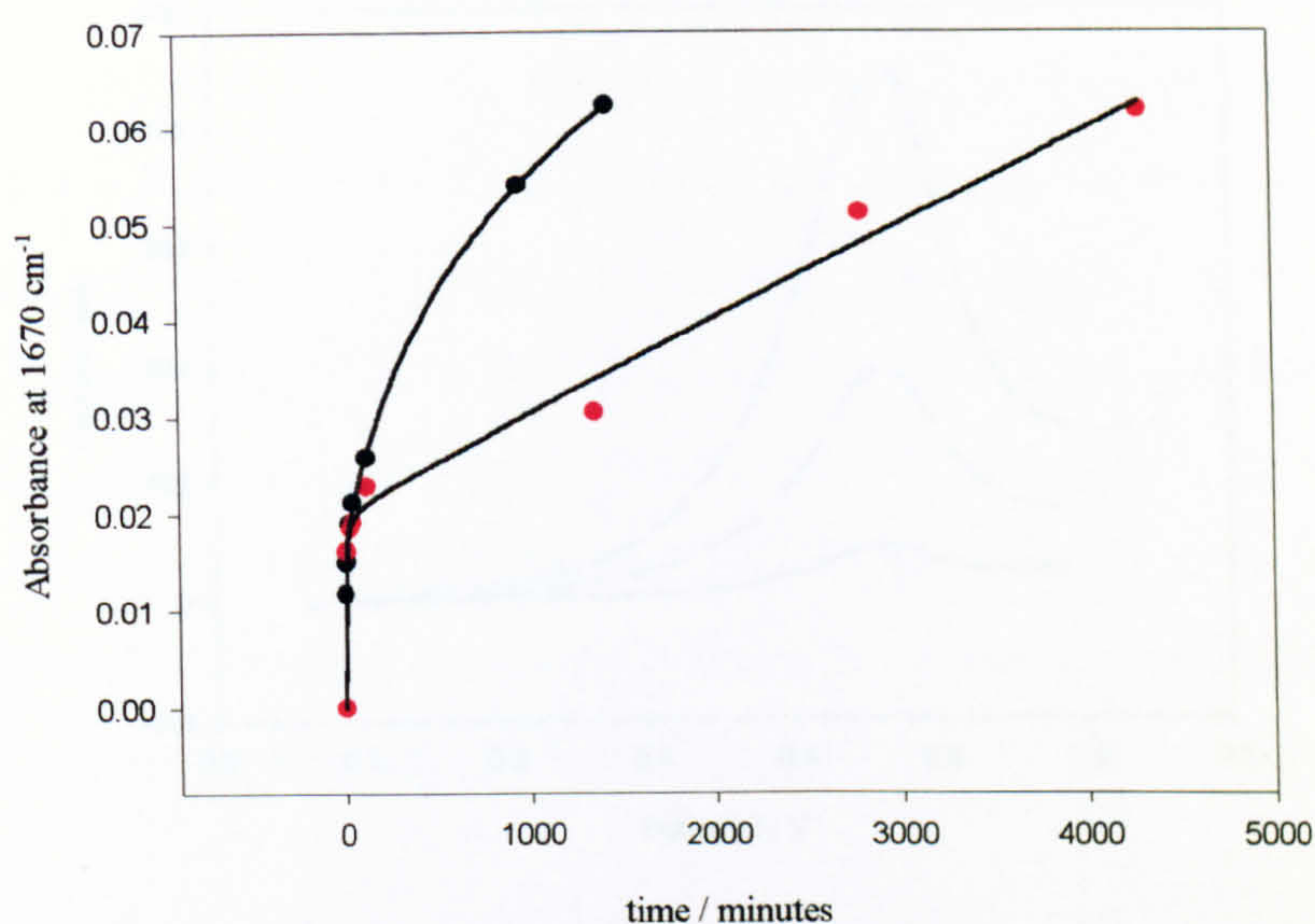
The cathodic current maximum was measured for each of the samples for both the fast and slow grown polymers and plotted against time in Figure 4.16. It is obvious from the results that current response of the fast grown polymer reaches a higher maximum than that of the slow grown film. This is also a consequence of the difference in microstructure of the films leading to a change in the influx of ions into the polymers. Although slow diffusion in both films results in minutes/hours being necessary for complete reaction, the migration driving force behind electroneutrality requirements means that redox cycling occurs on a timescale of seconds. Due to the porosity of the fast grown homopolymer it is able to allow the ions required for electroneutrality to migrate more readily through the film thereby causing an increased electrochemical response. The increase in electrochemical current for the fully reacted, fast grown films is very large. This may relate to the extent of diffusion on the timescale of voltammetry.



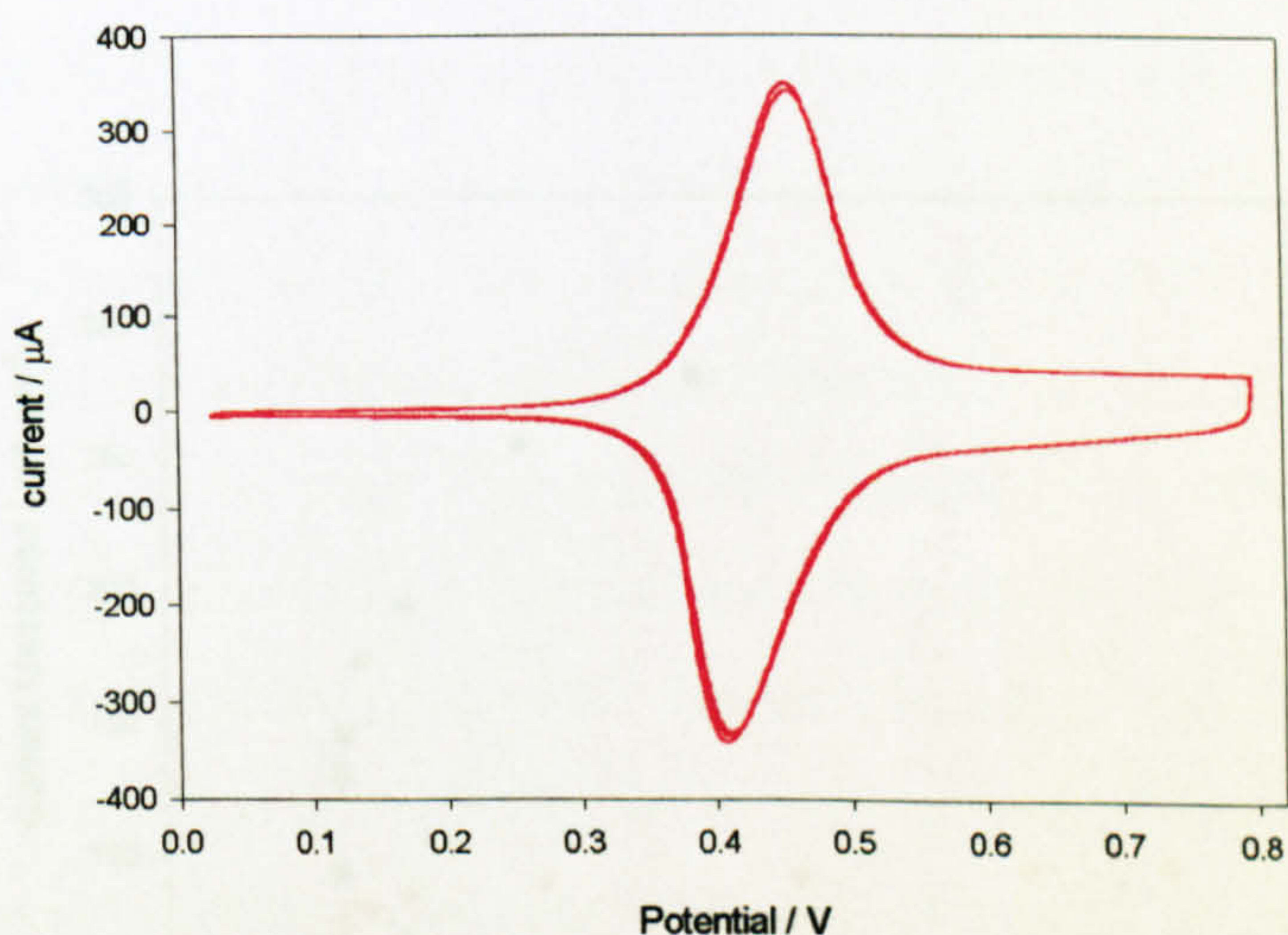


**Figure 4.12:** FT-RAIRS comparison of the rate of decrease in C=O absorbance of (a) fast (black) and (b) slow grown (red) poly(PFP) during the course of reaction with 0.1 mM ferrocene ethylamine and DMSO. It can be observed that the initial stage of reaction, both films react at the same rate. The reaction rate then slows down with fast grown reaching completion first.





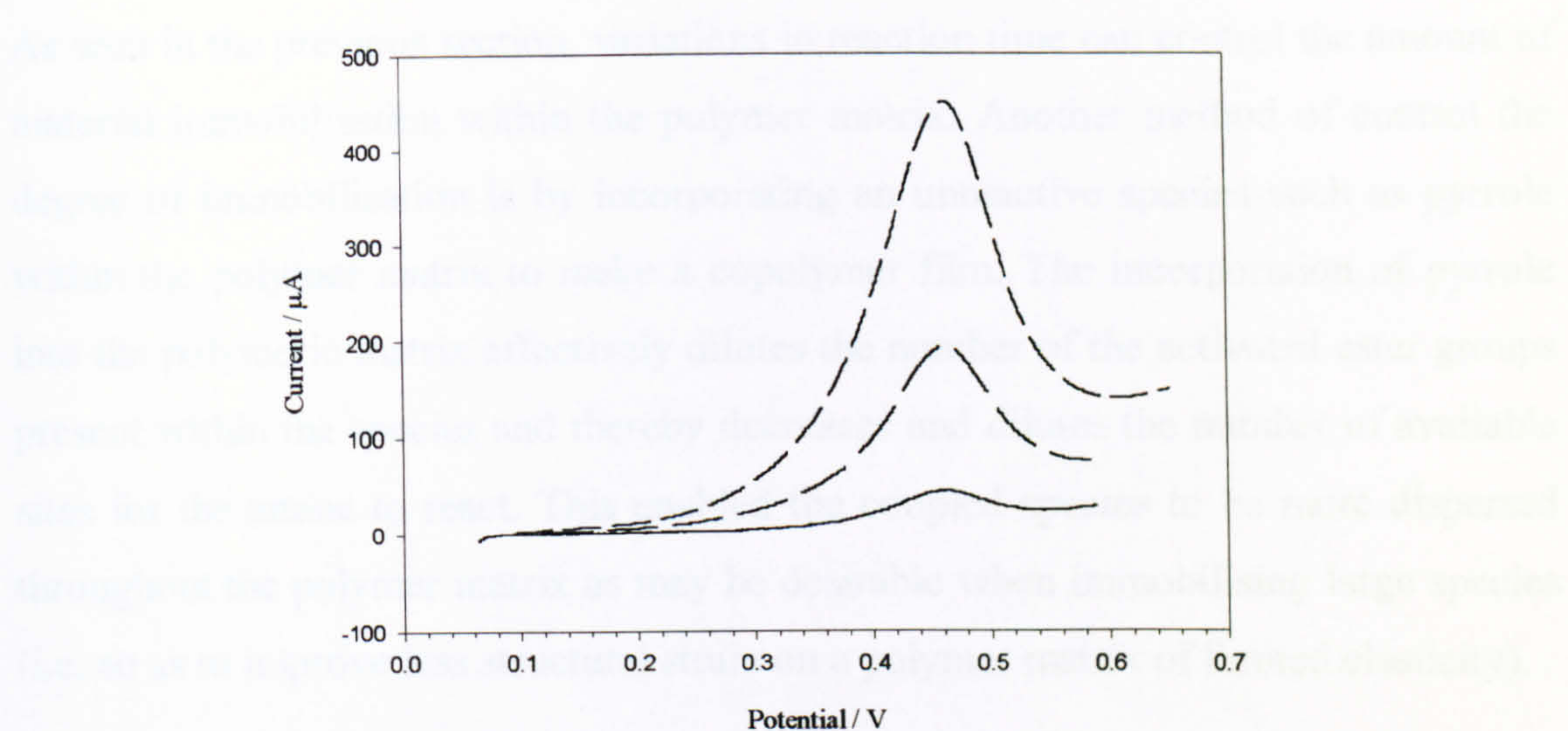
**Figure 4.13:** FT-RAIRS graph indicating the increase in absorbance at  $1670\text{ cm}^{-1}$  (formation of amide bond) for a fast (black) and slow (red) grown poly(PFP) film during the course of reaction with ferrocene ethylamine / DMSO solution.



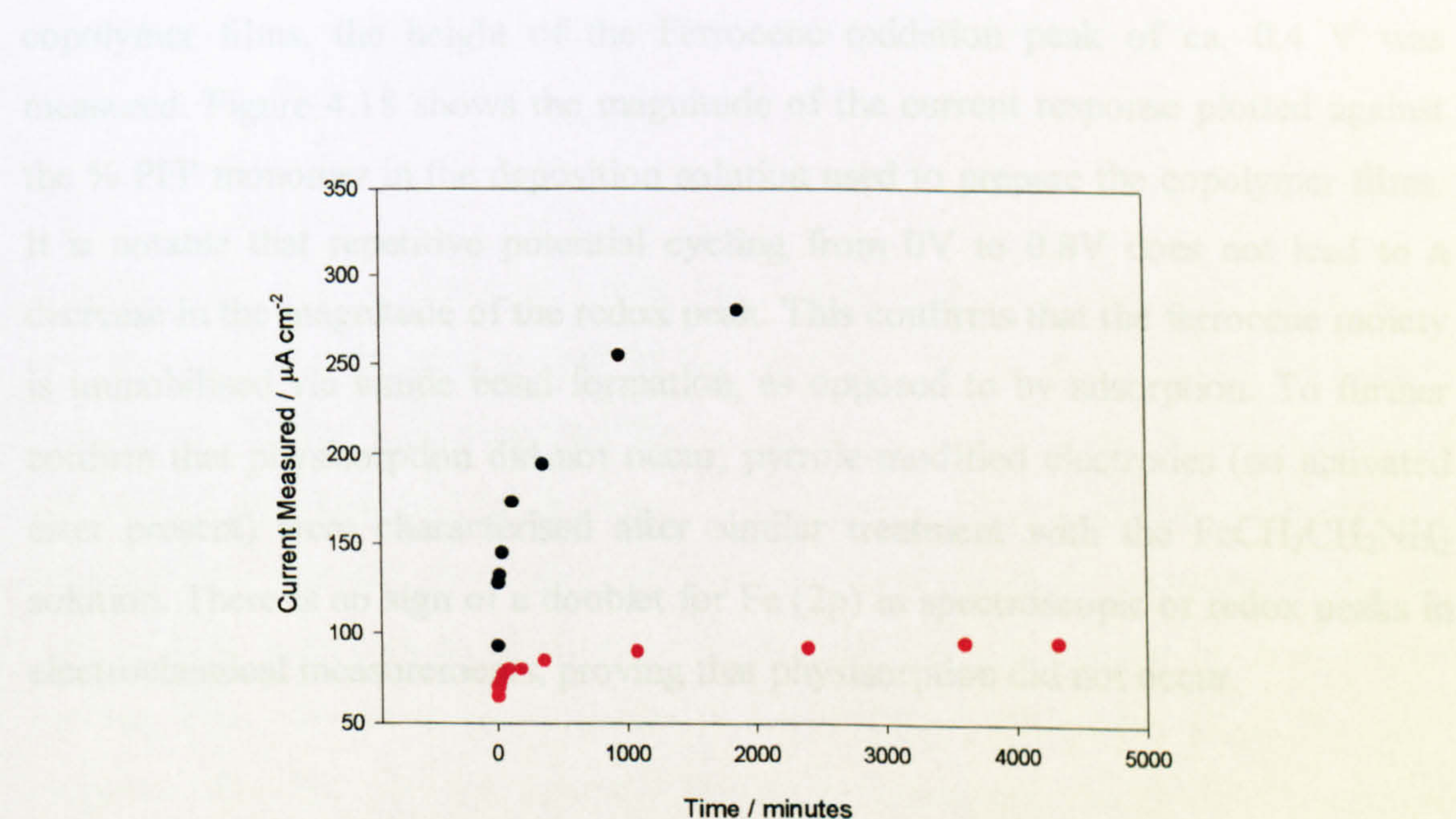
**Figure 4.14:** Cyclic voltammogram of poly(PFP) after complete reaction with ferrocene ethylamine and DMSO solution. Potential was scanned between 0 and 0.8V versus Ag|AgCl reference electrode; electrode area  $1\text{ cm}^2$ , supporting electrolyte 0.1 M solution of TEAP in acetonitrile and scan rate of  $20\text{ mVs}^{-1}$ . Redox peaks can be seen at 0.42V.



## 4.2.1 Synthesis of PTFPy Copolymer Films with Ferrocene Ethylamine



**Figure 4.15:** The variation of current with voltage scan rate for a reversible electron transfer reaction. The graph illustrates the oxidation sweep of a ferrocene ethylamine modified poly(PFP) film at scan rates;  $5 \text{ mVs}^{-1}$  (solid line),  $20 \text{ mVs}^{-1}$  (long dash line) and  $50 \text{ mVs}^{-1}$  (short dash line) when scanned in background solution of  $0.1 \text{ M TEAP}$  and  $\text{MeCN}$ . Electrode area =  $1 \text{ cm}^2$ .



**Figure 4.16:** Comparison of the cathodic current response of (a) fast grown (black) and (b) slow grown (red) poly(PFP) reacted with ferrocene ethylamine and DMSO solution for different intervals until complete reaction has occurred.



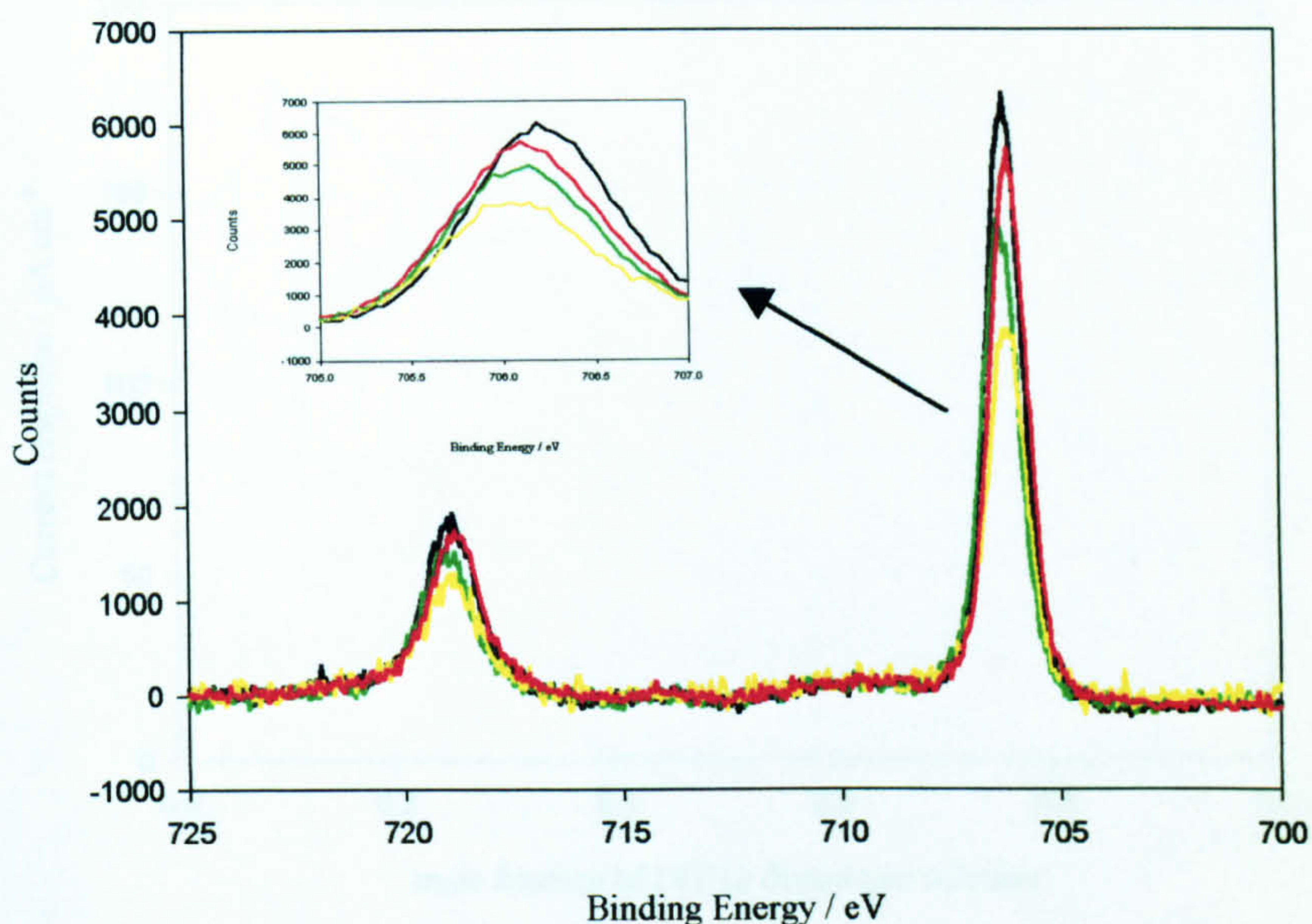
#### 4.2.1.3 Reaction of PFP/Py Copolymer Films with Ferrocene Ethylamine

As seen in the previous section, variations in reaction time can control the amount of material immobilisation within the polymer matrix. Another method of control the degree of immobilisation is by incorporating an unreactive species such as pyrrole within the polymer matrix to make a copolymer film. The incorporation of pyrrole into the polymeric matrix effectively dilutes the number of the activated ester groups present within the species and thereby decreases and dilutes the number of available sites for the amine to react. This enabled the coupled species to be more dispersed throughout the polymer matrix as may be desirable when immobilising large species (i.e. so as to improve less structural strain on a polymer matrix of limited elasticity).

From the XPS spectra of ferrocene ethylamine immobilisation on copolymer films (Figure 4.17) the amount of Fe (2p) detected by XPS decreases as the amount of PFP incorporated within the film is reduced (for ease copolymer compositions are referred to by the ratio of monomers within the deposition solution).

To assess the amount of electrochemically accessible  $\text{FcNH}_2$  for the different copolymer films, the height of the Ferrocene oxidation peak of ca. 0.4 V was measured. Figure 4.18 shows the magnitude of the current response plotted against the % PFP monomer in the deposition solution used to prepare the copolymer films. It is notable that repetitive potential cycling from 0V to 0.8V does not lead to a decrease in the magnitude of the redox peak. This confirms that the ferrocene moiety is immobilised via amide bond formation, as opposed to by adsorption. To further confirm that physisorption did not occur, pyrrole-modified electrodes (no activated ester present) were characterised after similar treatment with the  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solution. There is no sign of a doublet for Fe (2p) in spectroscopic or redox peaks in electrochemical measurements, proving that physisorption did not occur.

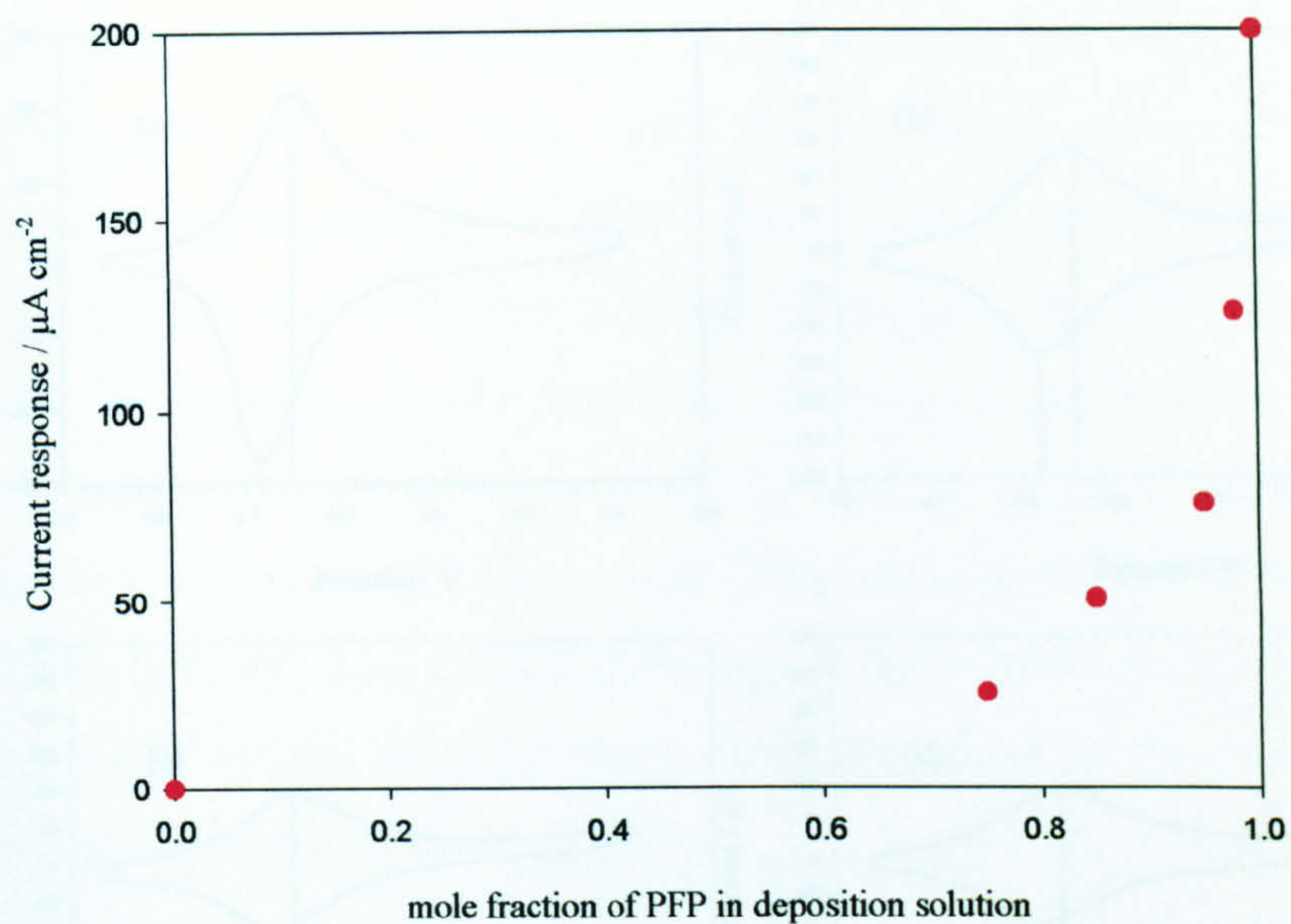




**Figure 4.17:** Fe (2p) XPS spectra (TOA 90 degrees) of homopolymer and PFP/ Py copolymers after reaction with Ferrocene Ethylamine and DMSO solution: % PFP: % Py in deposition solution used for electrosynthesis of polymers; (a) 100:0 (black), (b) 98:2 (red), (c) 95:5 (green), (d) 85:15 (yellow) (also containing 0.1 M TEAP and MeCN, electrode area 1 cm<sup>2</sup> and scan rate 20 mVs<sup>-1</sup>). The inset shows the region from 705 to 707 eV.

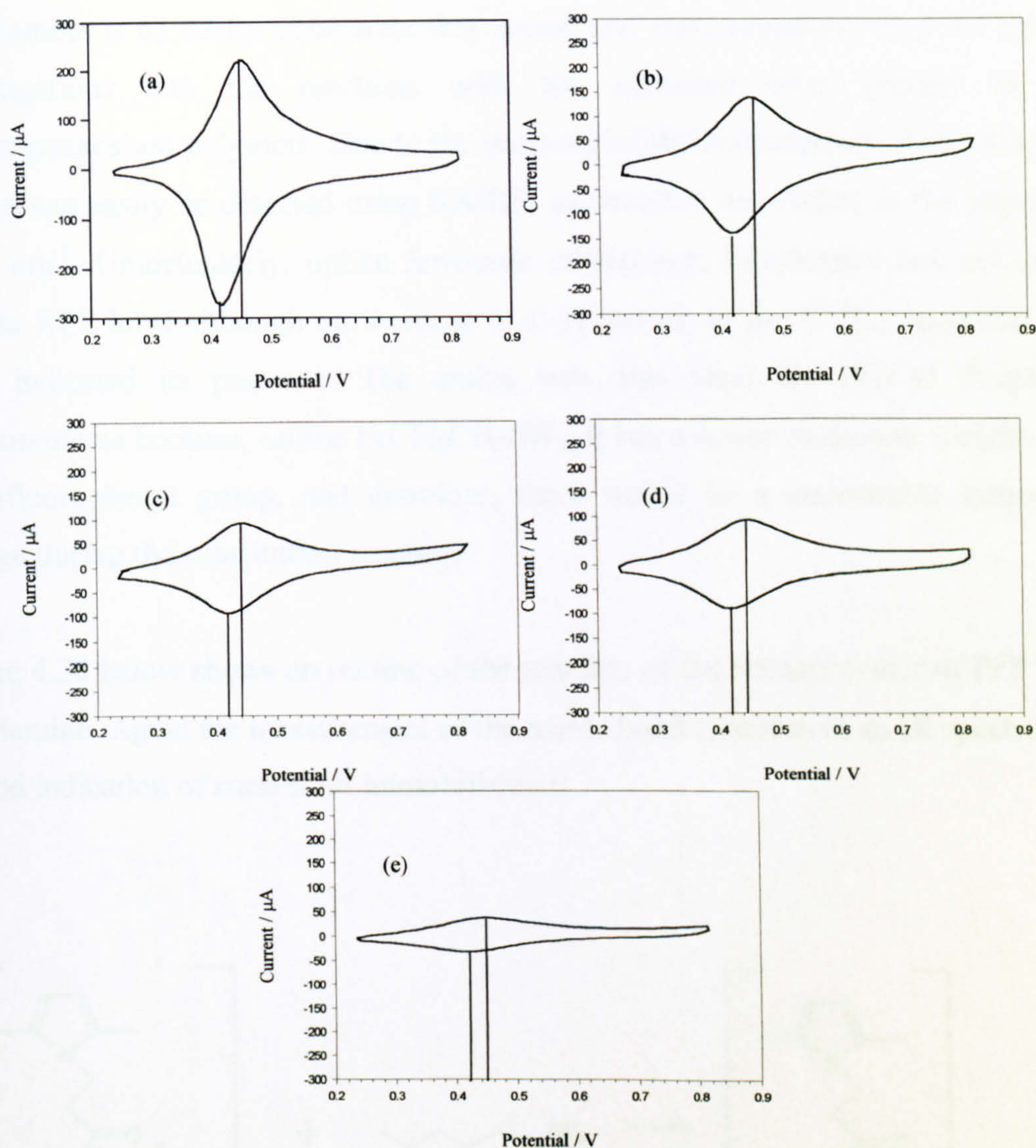
Significantly, it was observed from the cyclic voltammograms of the reacted copolymers that there was a shift in the positions of the redox peaks for certain copolymers. Figure 4.19 illustrates the ferrocene redox switching for a sequence of copolymers and show that the incorporation of pyrrole initially enhances the electron transport rate (smaller peak-peak separation). However, large amounts of pyrrole lead to both decreases in electron transport rate and the amount of immobilised species. This may be a consequence of ion transport limitations since the small numbers of redox sites are sparsely dispersed throughout the positively charged polymer and so the ionic capacity of the polymer is partially filled by perchlorate ions acting as the dopant for poly(pyrrole).





**Figure 4.18:** Graph showing the variation of current response of copolymers of PFP and pyrrole with PFP mole fraction of 1.0, 0.98, 0.95, 0.85 0.75 and 0 after reaction with 1 mM ferrocene ethylamine and DMSO solution.





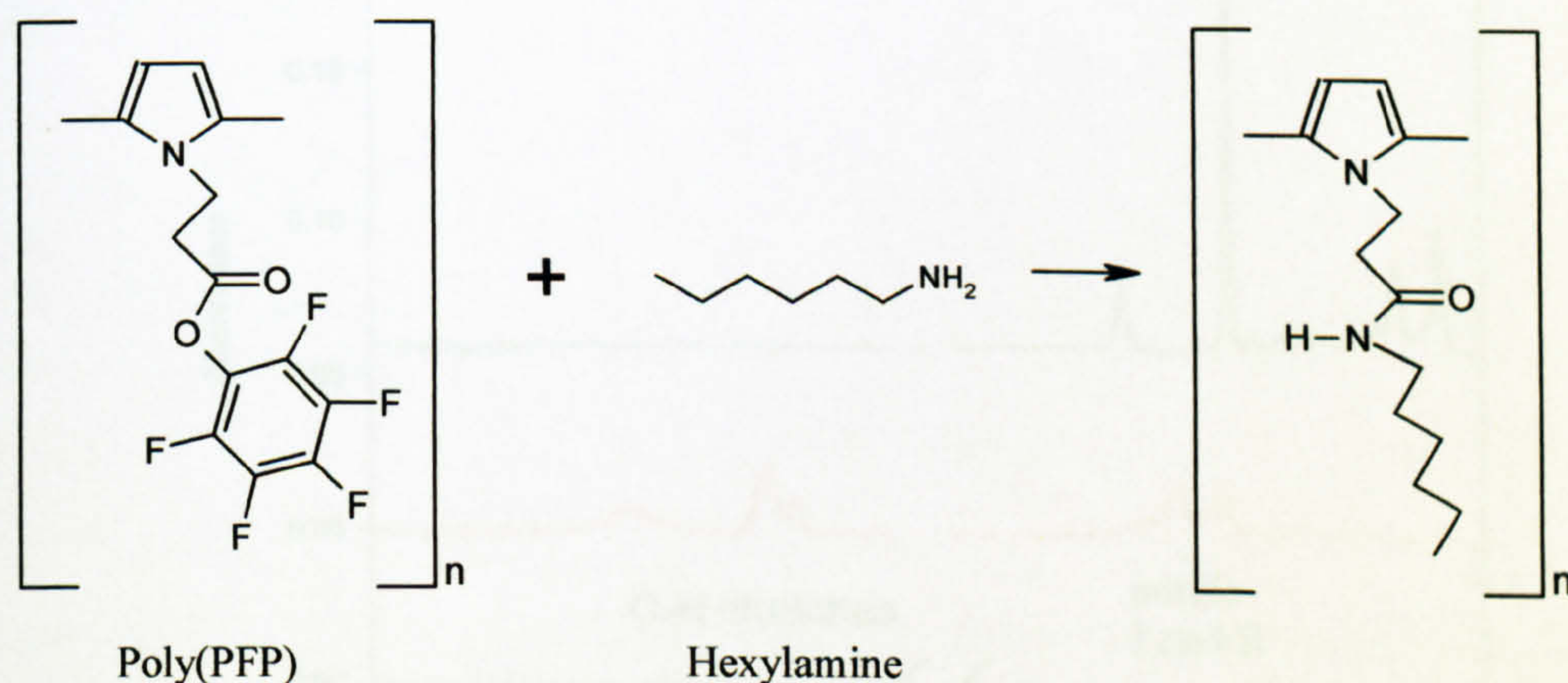
**Figure 4.19:** Cyclic Voltammograms of copolymer modified electrodes after reaction with ferrocene ethylamine, scanned in 0.1 M TEAP and MeCN solution. Copolymers were polymerised from deposition solution containing PFP: Pyrrole ratio of; (a) 100:0, (b) 98:2, (c) 95:5, (d) 85:15 and (e) 75:25. The approximate peak separations are; (a) 50 mV, (b) 30 mV, (c) 24 mV, (d) 30 mV and (e) 34 mV. Electrode areas = 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.



### 4.2.2 Reaction with Hexylamine

Hexylamine is an easily obtainable free amine and was, therefore, used for further investigations into the reactions with the activated ester present in the electrosynthesised polymers. Due to the presence of the hydrocarbon chain, its C-H groups can easily be detected using RAIRS, as stretches are visible in the region of  $3000\text{ cm}^{-1}$ . Unfortunately, unlike ferrocene ethylamine, hexylamine has not got a unique XPS label although an increase in C-H groups in the C (1s) spectrum was used indicated its presence. The amine was also ideal for EQCM frequency measurements because, unlike  $\text{FcCH}_2\text{CH}_2\text{NH}_2$ , it has a lower molecular weight than pentafluorophenyl group, and therefore, there would be a measurable frequency change during the substitution reaction.

Figure 4.20 below shows an outline of the reaction of the homopolymer of PFP with hexylamine. Again the measurement of the amide bond stretches in an IR spectrum is a good indication of successful immobilisation.

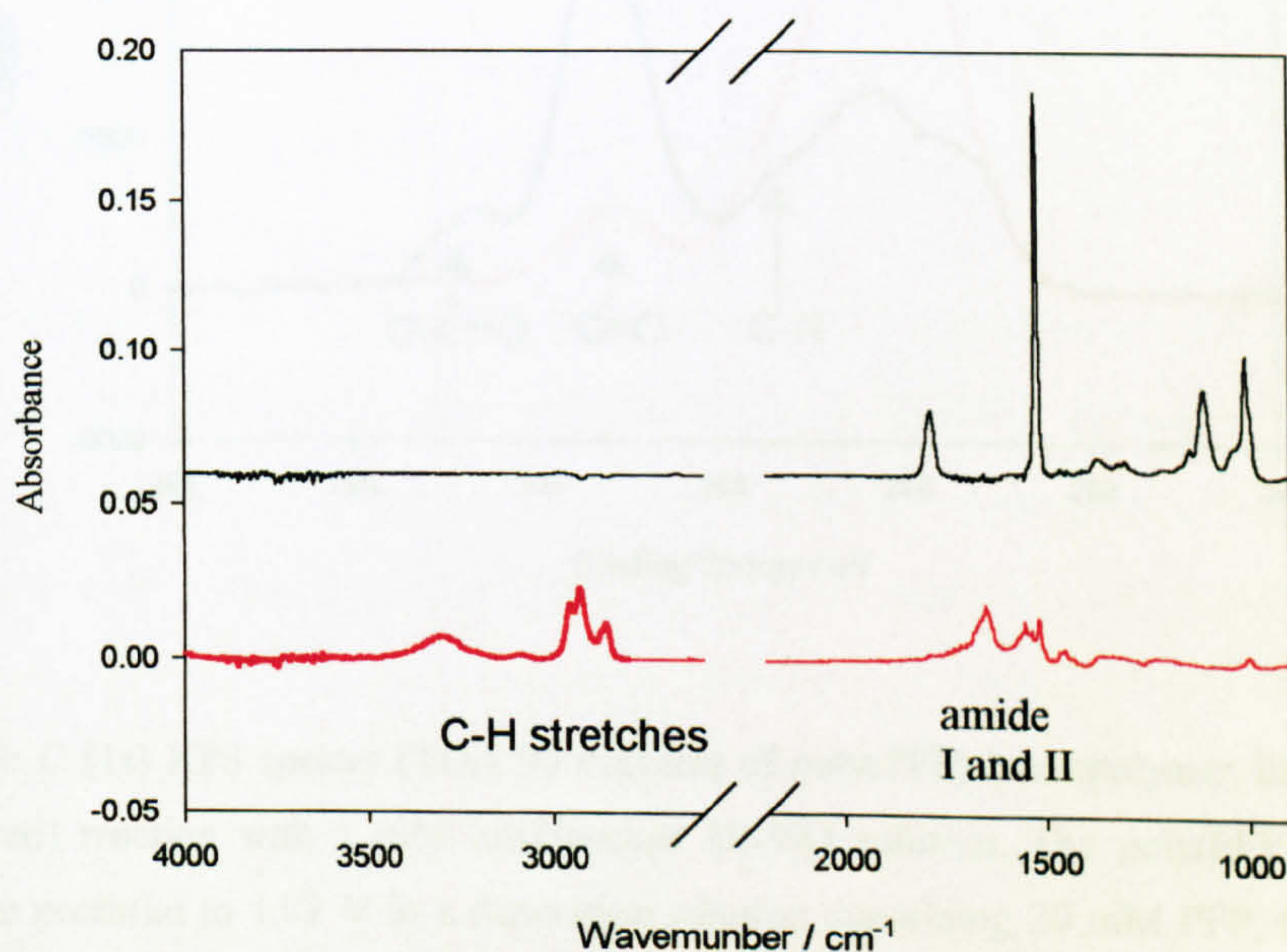


**Figure 4.20:** Scheme illustrating the reaction of poly(PFP) with hexylamine. The amine reacts with the activated ester of the polymer to produce amide bonds.



#### 4.2.2.1 Poly(PFP) Homopolymer Reaction with Hexylamine

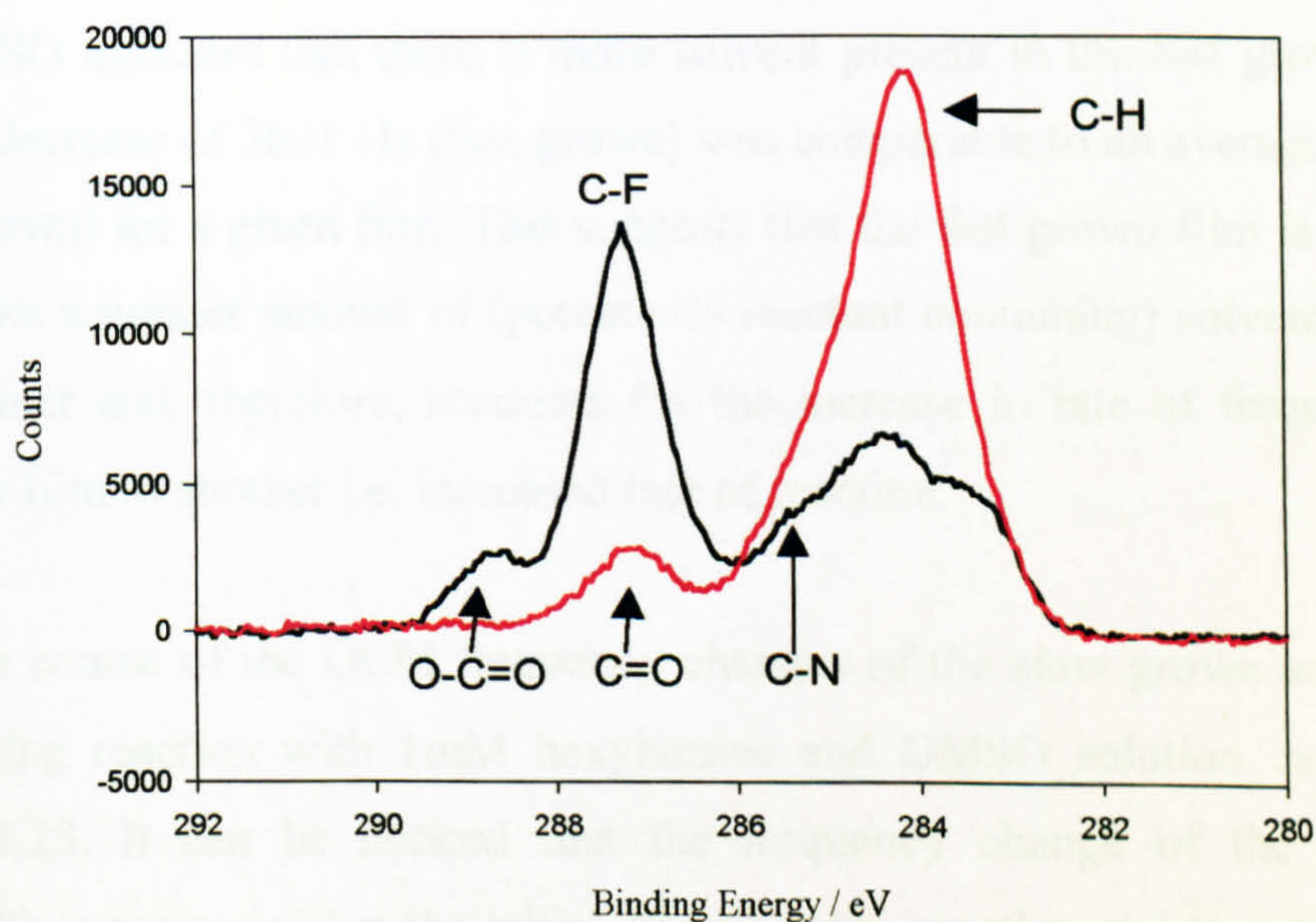
RAIRS analysis is collected as reflectance data and consequently converted into absorbance spectra as described in Chapter 2. The data obtained, as seen in Figure 4.21, again, show the specific peaks attributed to the attachment of the amine to the polymer film. This confirms that amide bond formation is the route of attachment of the hexylamine. The RAIRS spectra include that of the reference unreacted poly(PFP) and also the presence of the amide bond after reaction (again no hydrolysis reaction is apparent). Also obvious is the presence of the C-H stretches at 2850  $\text{cm}^{-1}$ , in the RAIRS spectrum, which is due to the presence of the alkyl chain of the hexylamine. As with ferrocene ethylamine, the reaction of hexylamine with the activated ester is limited by the rate of penetration throughout the film. Although in this case hexylamine molecule has a lower molecular weight and, therefore, can diffuse through the matrix at a faster rate and moreover can reach complete reaction within a shorter time limit, as was confirmed by EQCM results described below.



**Figure 4.21:** RAIRS absorbance spectra of unreacted PFP homopolymer before (black) and after (red) reaction with 1 mM hexylamine/ DMSO solution. The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1  $\text{cm}^2$  and a scan rate of 20  $\text{mVs}^{-1}$  was used.



As indicated hexylamine does not provide a good source to allow easy detection using XPS. However, the immobilisation of alkyl chains to the activated ester of the polymeric film causes an increase in C-H and C-N groups present in the polymer. It is not possible to quantify the amount of hexylamine incorporated in the polymer due to the presence of C-H and C-N groups already within the unreacted homopolymer. However by comparing the change in the number of counts detected in the specific regions it is possible to determine whether immobilisation has occurred. From Figure 4.22 it is possible to observe that there is an increase in the groups of interest compared to the unreacted reference polymer.



**Figure 4.22:** C (1s) XPS spectra (TOA 90 degrees) of poly(PFP) homopolymer film before (black) and after (red) reaction with 1 mM hexylamine/ DMSO solution. The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

When considering the reaction of hexylamine with the PFP polymer it is possible to follow the course of reaction using EQCM. This technique can measure the change

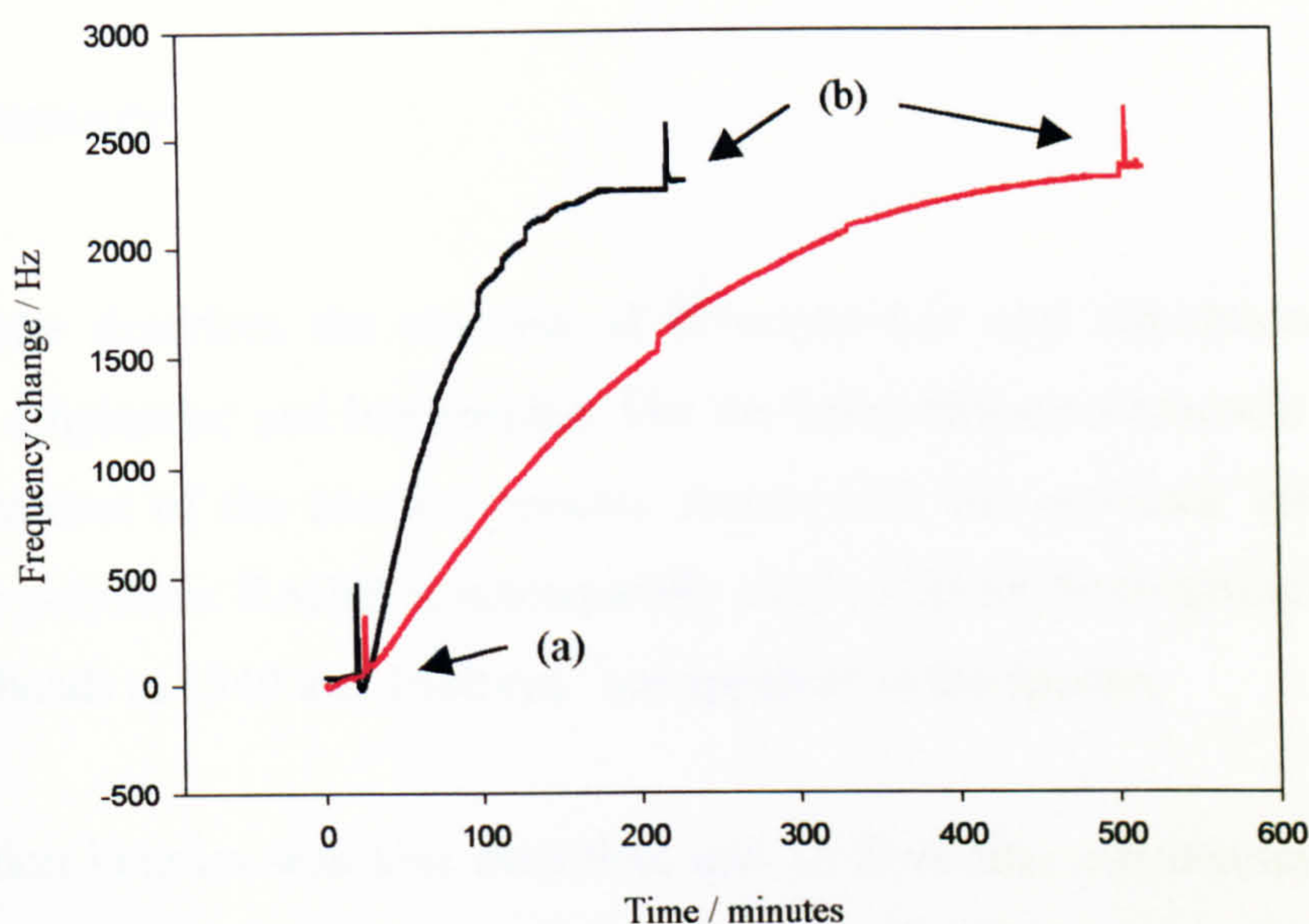


in mass of the polymer over the reaction period. Due to a significant difference in molecular weight of the nucleophilic hexylamine group and the leaving, pentafluorophenyl group of the original polymer, it is possible to measure the resultant mass changes due to the nucleophilic reaction. As previously, experiments were carried out using fast and slowly grown polymers to confirm whether changing the electrodeposition conditions did indeed affect the reaction rate with the unreacted polymers and explore an in situ method of monitoring the progress of the immobilisation reaction.

When preparing the fast and slow grown polymers, care was taken to produce polymer modified quartz crystals with the same quantity of poly(PFP) electrodeposited on them. Confirmation of this is possible by calculating the dry mass of polymer from the frequency change produced after the growth process. Notably, the solvated frequency change, when both samples were placed in amine free DMSO indicates that there is more solvent present in the fast grown film. The average decrease of 3601 Hz (fast grown) was comparable to an average of 988.2 Hz (slow grown) for a given film. This suggests that the fast grown film is more porous and allows a greater amount of (potentially reactant containing) solvent to soak into the polymer and, therefore, accounts for the increase in rate of frequency change from one film to another i.e. increased rate of reaction.

The time course of the QCM frequency changes of the slow grown and fast grown PFP during reaction with 1mM hexylamine and DMSO solution can be seen in Figure 4.23. It can be noticed that the frequency change of the faster grown poly(PFP) is more rapid at the initial stages of the reaction compared to that of the slower grown poly(PFP). Furthermore, validating previous results, the fast grown poly(PFP) reaches complete reaction before the slow grown polymer (completion of reaction was confirmed by using FTIR to detect the disappearance of ester stretch and formation of the amide bond stretches). Amide bond absorbance bands are the same for both films showing that both polymers were of similar thickness.





**Figure 4.23:** QCM frequency change (mass decrease) for fast grown (black) and slow grown (red) poly(PFP) coated electrodes immersed in 1 mM hexylamine and DMSO solution where (a) is reactant solution addition and (b) is background solution addition. Note that polymer films grown on quartz crystal electrodes (area  $0.229 \text{ cm}^2$ ) using the same deposition conditions to compare the thickness of each film before the experiments was carried out. It was seen that both films were approximately 120 nm using dektak measurements.

For the hexylamine (HA) reaction ( $\text{poly(PFP)} + \text{HA} \rightarrow \text{Py-HA}$  ( $303 \text{ g mol}^{-1}$  to  $221 \text{ g mol}^{-1}$ )) the overall change in mass of the unsolvated polymer film following complete reaction would correspond to a decrease of 27 %. For the fast grown polymer film it took, approximately 180 minutes to reach complete reaction and for the slow grown film approximately 480 minutes. Furthermore hexylamine modified polymer films immersed in reactant free DMSO show no significant change in solvated polymer frequency. This measurement therefore provides the indication that DMSO does not cause any changes in the polymer composition (e.g. hydrolysis). This can be confirmed by measuring the FTIR spectrum of the polymer after soaking with DMSO (no significant changes are observed in the spectrum). As previously mentioned the change in rate of reaction suggests that there is a change in



morphology/ porosity when different rates of growth of polymers are used for electrosynthesis of the films.

### ***4.3 Summary***

This chapter describes the reaction of homopolymer and copolymer films with ferrocene ethylamine and hexylamine. For the immobilisation reaction to occur the amine terminus of the reactive species reacts with the activated ester contained within the polymers. RAIRS is subsequently used to detect the resulting amide bonds as amide bands at 1540 and 1680  $\text{cm}^{-1}$  are apparent in the spectra.

The reaction kinetics was also described and as ferrocene ethylamine has an XPS probe it was used for the experiments performed. Using low concentration of ferrocene ethylamine, results show that it takes approximately 30 minutes for the polymer surface to completely react. By using RAIRS to monitor the amide bond formation it was noted that for complete reaction to occur throughout the bulk of the film it takes approximately 1440 minutes (time needed for diffusion throughout film). Moreover, as the film thickness increases from 25 nm to 100 nm the reaction time increases from 60 minutes to 4320 minutes. It was also noted that slow grown and fast grown homopolymer films react with ferrocene ethylamine at different rates. The slow grown film is completely reacted in 4320 minutes and the fast grown film takes approximately 1440 minutes. This emphasises the importance in using the same growth conditions to produce polymer-modified electrodes for further experiments.

A second method used to assay the immobilisation reaction was to use the electrochemical response detectable due to the ferrocene centre. It was shown that completely reacted fast grown films have a higher current response than the slow grown film. Again this is a result of a difference in microstructure between the two films leading to a change in the influx of perchlorate ion into the film.



Furthermore, using Py/PFP copolymer films can also control the amount of ferrocene immobilised within the film. As would be expected, as the amount of pyrrole incorporated within the film increases the amount of ferrocene immobilised decreases. This was illustrated using both XPS and electrochemical results.

As ferrocene ethylamine was not suitable for EQCM experiments, hexylamine was used to monitor the immobilisation reaction using EQCM. These experiments show that when hexylamine reacts with poly(PFP) the frequency measurements increase as the leaving PFP group has a higher molecular weight than hexylamine. Again it was noted that there was a change in reaction rates between fast and slow grown poly(PFP) film.

## 4.4 References

1. Godillot, P., Korri-Youssouffi, H., Srivastava, P., El Kassmi, A., Garnier, F., "Direct Chemical functionalization of as-grown electroactive polypyrrole film containing leaving groups", *Synthetic Metals*, vol. 83, pp. 117, 1996.
2. Korri-Youssouffi, H., Godillot, P., Srivastava, P., El Kassmi, A., Garnier, F., "New method of polypyrrole functionalisation toward molecular recognition", *Synthetic Metals*, vol. 84, pp. 169, 1997.
3. Schuhmann, W., "Electron-transfer pathways in amperometric biosensors. Ferrocene-modified enzymes entrapped in conducting-polymer layers", *Biosensors and Bioelectronics*, vol. 10, pp.181, 1995.
4. Hudson, R., "Ferrocene polymers: current architectures, syntheses and utility", *Journal of Organometallic Chemistry*, pp. 637, 2001.
5. Lednicer, Lindsay, Hauser, "Synthesis of  $\beta$ -ferrocene ethylamine", *Journal of Chemical Society*, vol. 23, no. 5, pp. 653, 1958.

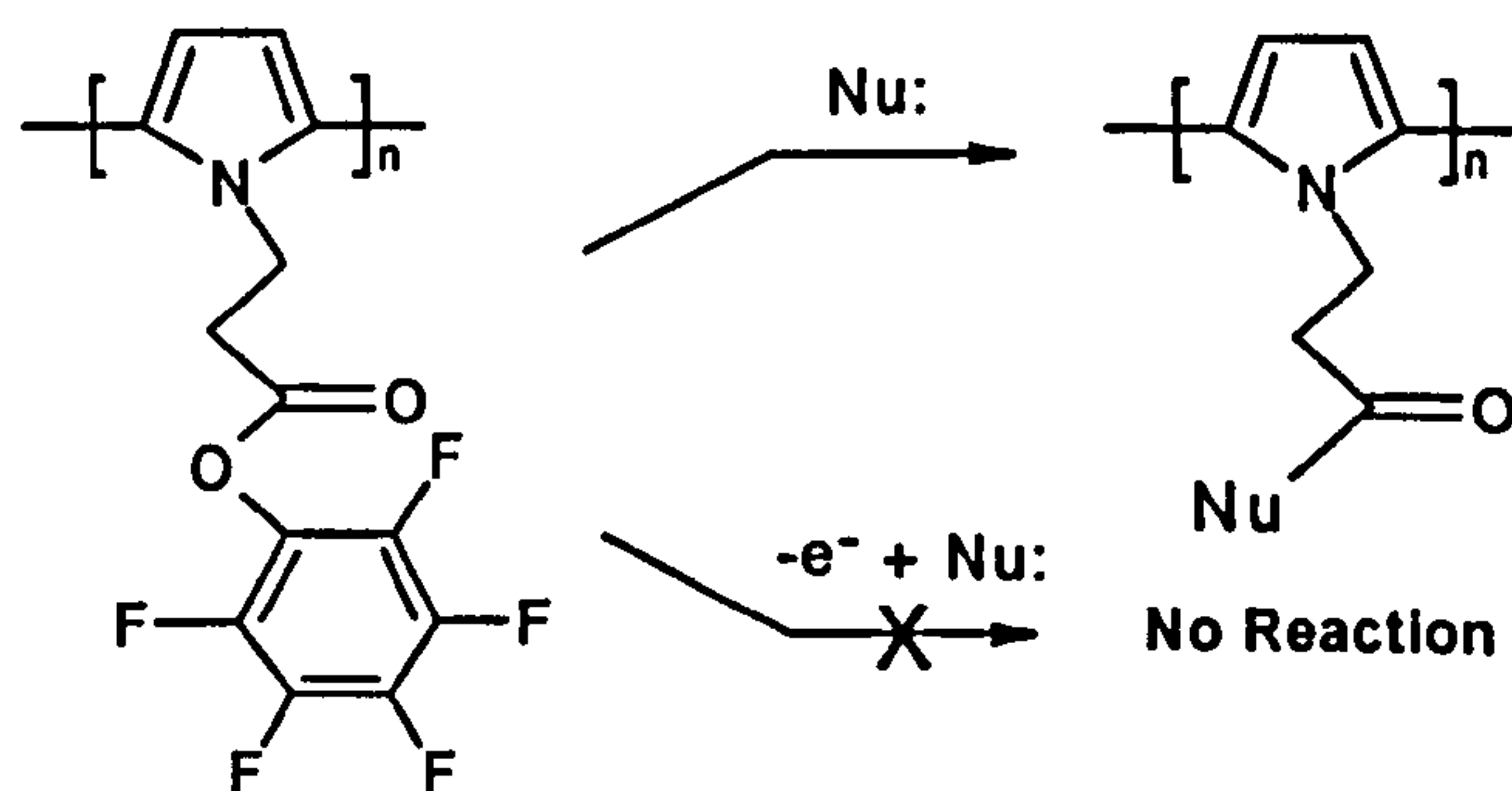


# Chapter 5

## *Development of a Method to Control the Modification of Poly(PFP) Electrodes*

### *5.0 Introduction*

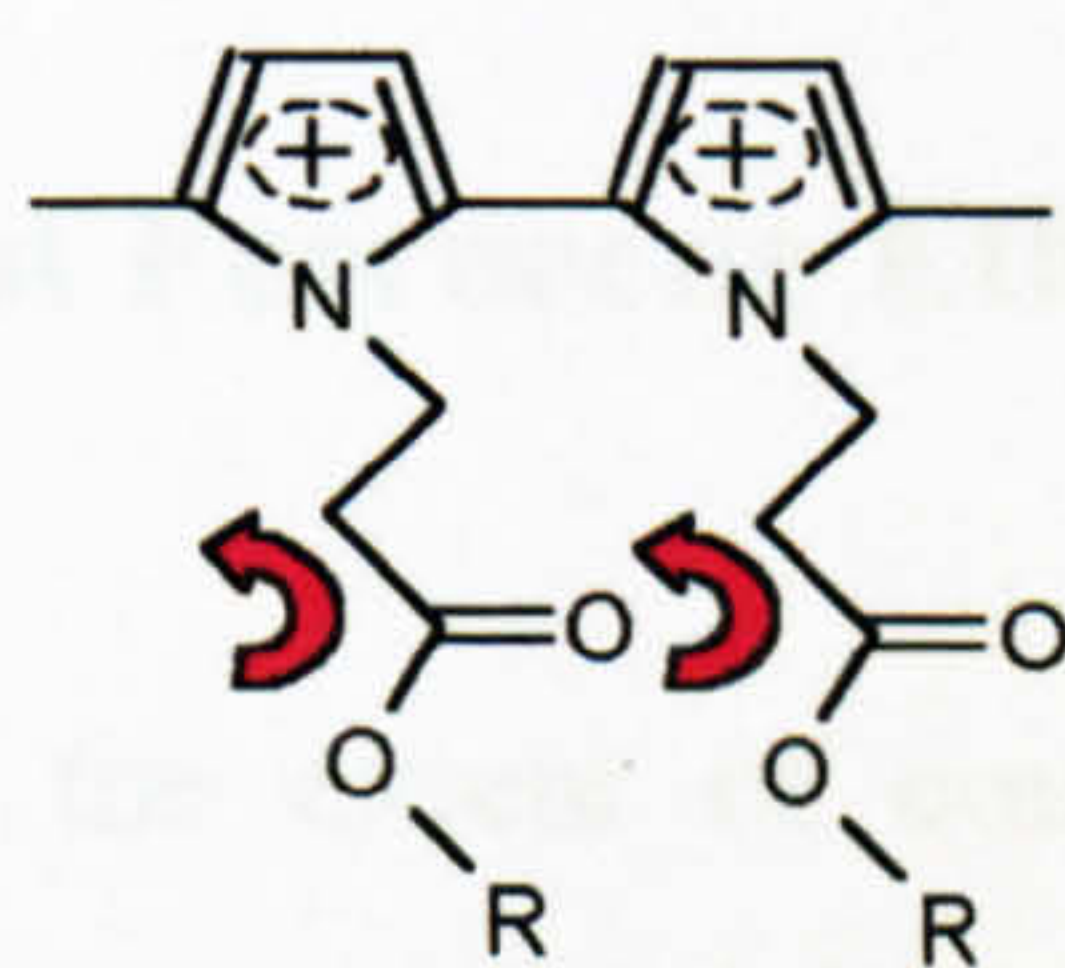
Previous methods have been described illustrating that it is possible to selectively attach different biological species in precise areas of a surface by using a combination of self-assembly and photo-activation<sup>1-3</sup>. Significantly, a new electrochemical method to inhibit/ promote the reaction of simple amines with the homopolymer of PFP is described in this thesis, and is illustrated in Figure 5.1<sup>4</sup>. It was found that there was reduced reaction of the simple amines with the oxidised homopolymer of PFP. This method is used in a series of experiments to micropattern various species on a multiple electrode device and an interdigitated electrode. The experiments involved using material, such as ferrocene ethylamine and MP-11, each with distinct spectroscopic probes to enable easy detection of their presence within the polymer modified electrodes using the XPS imaging facility.



**Figure 5.1:** Reaction scheme showing that doping the poly(PFP) film stops reaction with nucleophilic species ( $\text{Nu:}$ ) and successful reaction with undoped polymer films.



Underpinning the proposal scheme was the hypothesis that by maintaining the polymer in the oxidised (doped) state, the electronic distribution in the activated ester group would be polarised towards the polymer backbone, rather than the pentafluorophenol leaving group. This polarisation would reduce the effectiveness of the leaving group and thus lower the reactivity towards nucleophilic substitution. It was also noticed (see Chapter 5, section 5.2.3) from experiments, using amine solutions containing 0 M, 0.1 M and 0.2 M TEAP that the rate of substitution reaction decreases when the electrolyte concentration increases (for example, when using the 0.2 M TEAP solution the reaction rate decreases dramatically, and therefore, only a limited substitution occurred). This concentration mimics the doped state of the poly(PFP) backbone, therefore, this suggests that the presence of salt effects the reaction rates of poly(PFP) with the nucleophile<sup>5</sup> (see Section 6.2.3 in Chapter 6).



**Figure 5.2:** Schematic diagram indicating suggested direction of the flow of electrons (red arrows) when poly(PFP) is in an oxidised (doped) state. R represents the remaining part of the PFP leaving group.

To investigate this effect, electropolymerised films of poly(PFP) were immersed in either dry, or semi-aqueous dimethyl sulphoxide or acetonitrile based solutions containing both supporting electrolyte (TEAP) and an amine containing nucleophilic species.



## ***5.1 Materials***

**Solvents:** DMSO (99+%, anhydrous) and MeCN (99.5+%, ACS reagent) were obtained from Aldrich.

**PFP and TEAP** were prepared as described in Chapter 2 (Materials Section 2.1)

**Ferrocene Ethylamine:** prepared using method described in Chapter 4

**Hexylamine and MP-11** were obtained from Sigma-Aldrich.

**Interdigitated Electrodes:** prepared using protocol in Chapter 2.

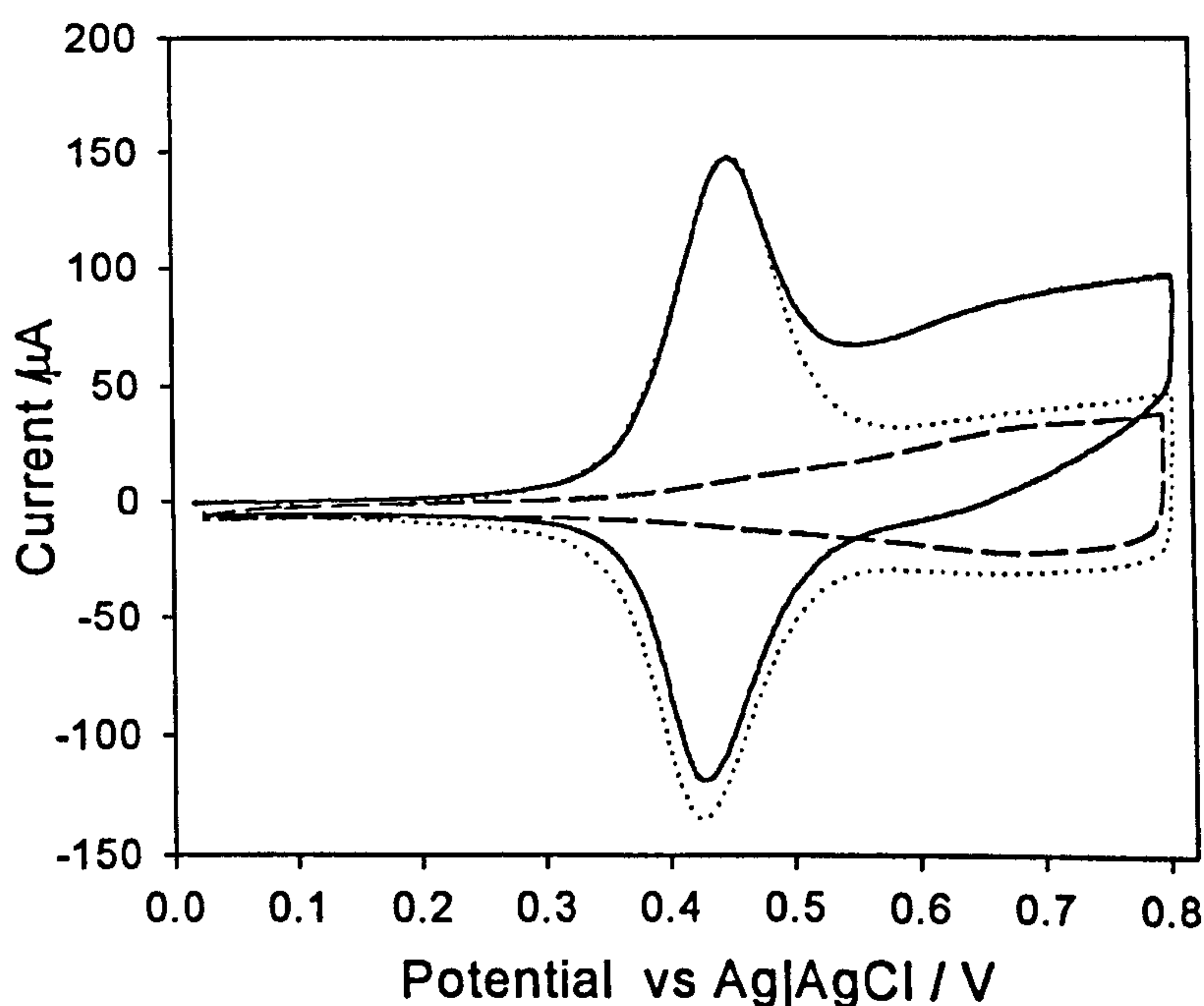
## ***5.2 Results and Discussion***

### **5.2.1 Control of Reaction of Ferrocene Ethylamine with Poly(PFP).**

To facilitate the evaluation of the extent of coupling, the reactive nucleophilic species, ferrocene ethylamine, was used for initial investigations. Since this species contains a redox moiety, this enabled a ready evaluation of the extent of coupling under various conditions of solution and electrochemical control using XPS, RAIRS and electrochemical methods. The cyclic voltammograms in Figure 5.3 illustrate the degree of coupling which occurs on poisoning a poly(PFP) electrode in a 1 mM  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solution at either 0V, or 0.77 V for 3 minutes. In addition, the voltammogram arising from the electrode previously held at 0.77 V in a  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solution, after re-immersion in the  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  for a further 3 minutes at 0V. These results clearly show the incorporation of ferrocene species into the membrane is inhibited by maintaining the polymer in a doped state as there is no visible redox response for the polymer. Furthermore, this inhibition is removed following the undoping of the same polymer.



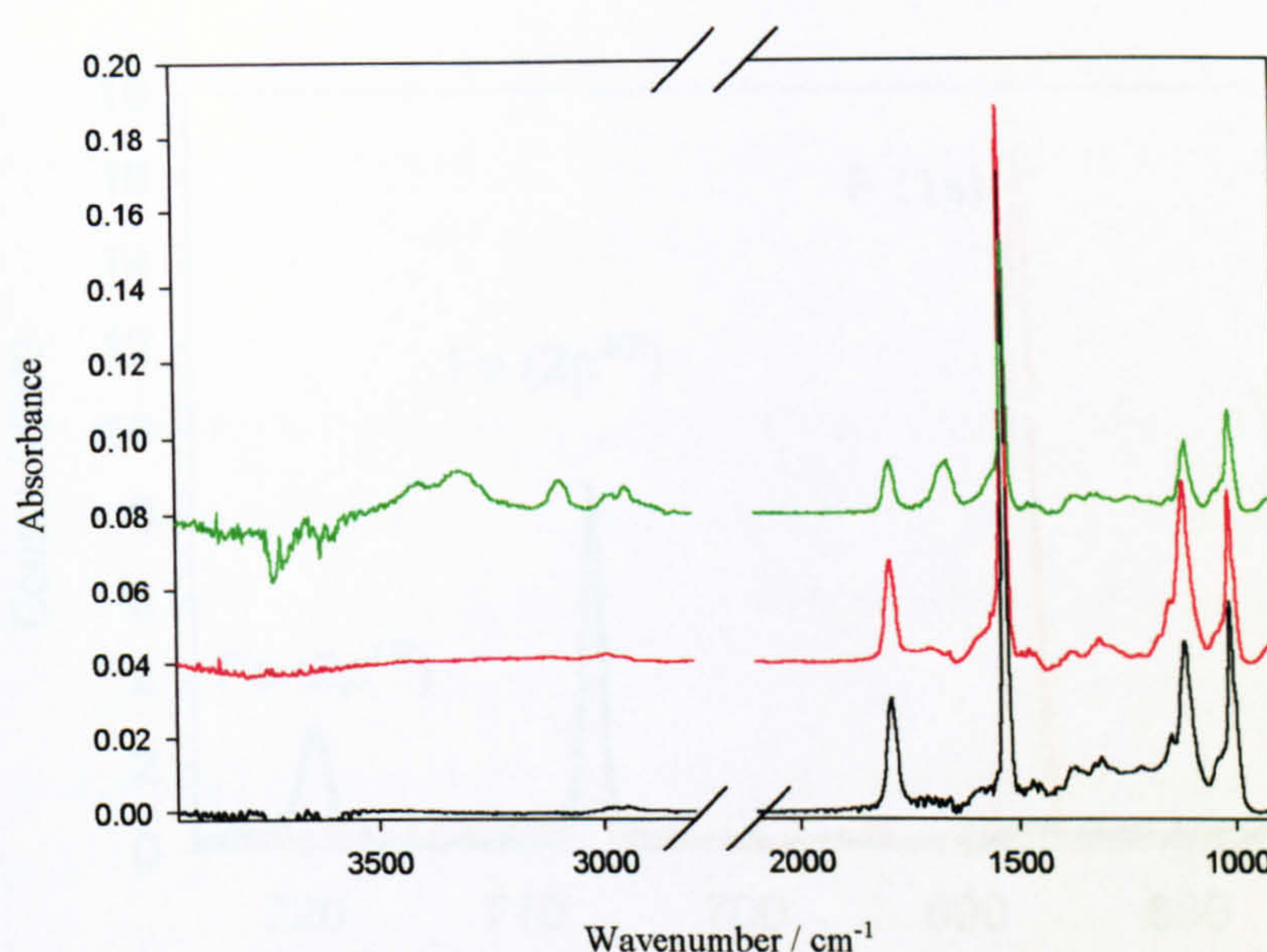
Taken by themselves, the results of Figure 5.3 do not necessarily indicate that the ferrocene species has become covalently attached to the polymer as a result of nucleophilic substitution. However, Figure 5.4 shows the RARS absorbance spectra for the as deposited poly(PFP) film, together with poly(PFP) electrodes which have been held in  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solutions at either 0 V, or 0.77 V for 3 minutes. The spectra of the film held in the  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  solution at 0 V show the loss of the fluorophenol ester peak at  $1790\text{ cm}^{-1}$  and with the appearance of amide I and amide II peaks, at  $1540$  and  $1680\text{ cm}^{-1}$ . Moreover there was little change between the unreacted PFP spectrum and the spectrum of the polymer held in the solution at 0.77V. Thus, it was concluded that the ferrocene present in the film after the appropriate electrochemical reaction conditions has been immobilised via the anticipated nucleophilic substitution.



**Figure 5.3:** Cyclic Voltammograms of poly(PFP) films after immersion in solution of 1 mM  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  and DMSO for 3 minutes in the following conditions of electrochemical control; (a) electrode held at 0 V (solid line), (b) electrode held at 0.77 V (dashed line) and (c) electrode held at 0.77 V in the amine solution then held at 0 V in the same solution (dotted line). CVs were obtained in solution containing 0.1 M TEAP and acetonitrile, the electrode areas were  $1\text{ cm}^2$  and the scan rate  $20\text{ mVs}^{-1}$ .



In addition, the Fe (2p) and F (1s) XPS spectra of Figure 5.5 show that the amount of ferrocene in the surface layers of the film following immersion of an electrode poised at 0.77 V in the ferrocene solution is negligible. This low level could not directly be inferred from the lack of signal in the corresponding CV since physisorbed ferrocene ethylamine, which could have been present, may have exhibited poor electronic communication with the poly(PFP) matrix.

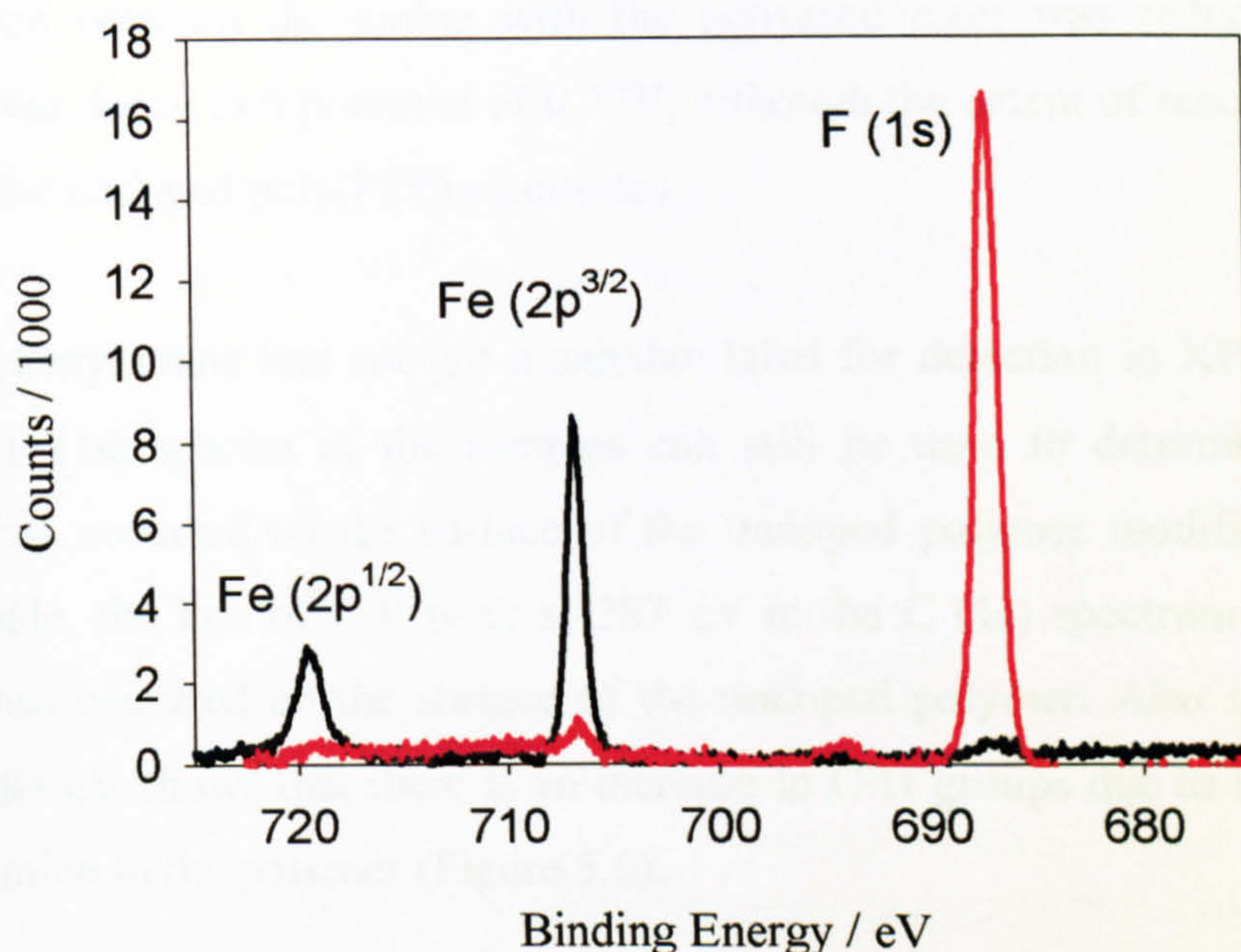


**Figure 5.4:** RAIRS absorbance spectra for poly(PFP) films unreacted and after immersion in 1 mM  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  and DMSO solution at specified electrochemical conditions: (a) unreacted poly(PFP) film (black), (b) oxidatively doped (at 0.77 V) poly(PFP) (red), (c) undoped poly(PFP) (green). The poly(PFP) was produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was  $1 \text{ cm}^2$  and a scan rate of  $20 \text{ mVs}^{-1}$  was used.

Since ferrocene ethylamine is in the positively charged, oxidised state at 0.77 V, investigations into whether this coupling inhibition was a consequence of membrane permselectivity to positive charged species were performed. In the previous example the polymer-modified electrodes were held at the oxidation potential when immersed



in the nucleophilic solution. Similar coupling experiments were performed but, in this second case, the homopolymer was doped and the electrode was not held at the potential during reaction (ferrocene ethylamine would therefore not be oxidised). Again, it was noticed that the reaction was inhibited when using the doped polymer, therefore, suggesting that permselectivity does not occur.



**Figure 5.5:** F (1s) and Fe (2p) XPS spectra (TOA 90 degrees) of poly(PFP) films after immersion in 1 mM FcCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, DMSO and 0.1 M TEAP solution for 5 minutes; polymer modified electrode (red) held (doped) at 0.77 V; undoped polymer modified electrode (black). The poly(PFP) was produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

As well as inhibiting aminolysis reaction, it was also found that the competing hydrolysis reaction was inhibited by application of an electrochemical potential. When the poly(PFP) modified electrode was immersed in basic semi-aqueous acetonitrile, DMF or DMSO solutions hydrolysis can be inhibited by electrode



polarisation at 0.77 V for approximately 30 minutes. When the undoped polymer electrode was immersed in the given solution it took less than 5 minutes for complete hydrolysis.

### 5.2.2 Control of Reaction of Hexylamine with Poly(PFP).

It was shown in Chapter 4 that stronger nucleophiles, e.g. aliphatic amines, show greater reactivity towards the poly(PFP). It was decided, therefore, to use hexylamine for further investigations of potential dependent coupling. Again results show that the reaction between the amine with the activated ester was reduced when the polymer was doped at a potential of 0.77V, although the extent of reaction remained the same for undoped poly(PFP) electrodes.

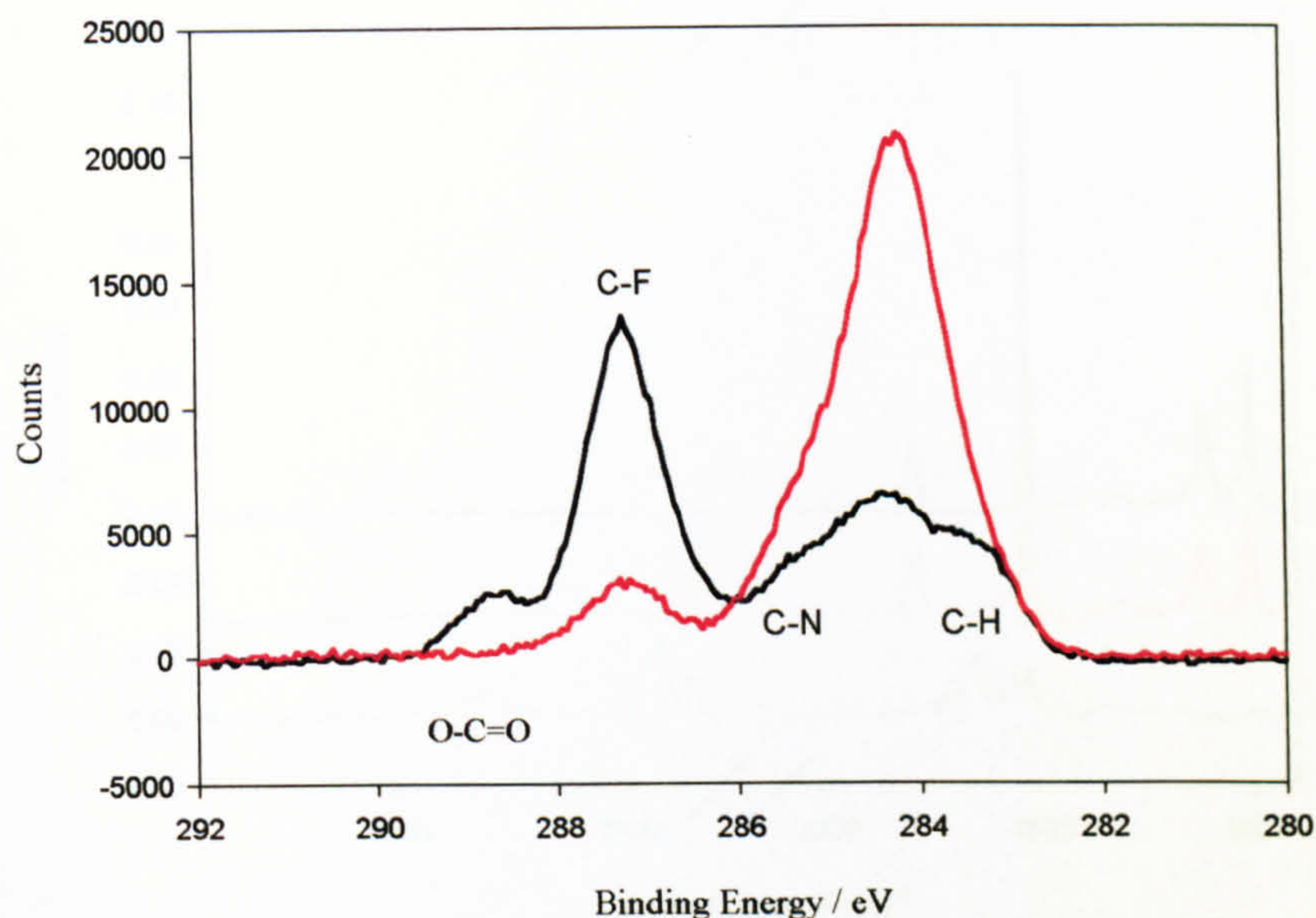
Although hexylamine has not got a suitable label for detection in XPS, both the C (1s) and F (1s) spectra of the samples can still be used to determine whether a reaction has occurred on the surface of the undoped polymer modified electrodes. For example, the loss of C-F peak at 287 eV in the C (1s) spectrum indicates that reaction has occurred on the surface of the undoped polymer. Also the increase in peak at 284 eV shows that there is an increase in C-H groups due to the attachment of hexylamine to the polymer (Figure 5.6).

By reducing the concentration of hexylamine in the solutions from 0.5mM to 0.1mM, the ratio of the coupling reaction at 0V to that at the inhibiting potential at 0.77V increases from approximately 5:1 to 10:1. This suggests that the electrochemical inhibition of the reaction with the polymer-modified electrodes can be optimised by using lower concentrations of amine in solution.

Figure 5.7 show the RAIRS spectra for the poly(PFP) held at 0V and 0.77V in 0.1mM hexylamine solution for 5 minutes. Again the spectrum indicates a limited reaction for electrodes held at 0.77V and complete reaction when poly(PFP) held at 0V. This conclusion is corroborated by the appearance of amide bands and C-H stretches at  $2850\text{cm}^{-1}$ , arising from the alkyl chain of hexylamine. Again it was found



that following removal of the potential after doping at 0.77 V, significant inhibition of the aminolysis reaction is maintained.

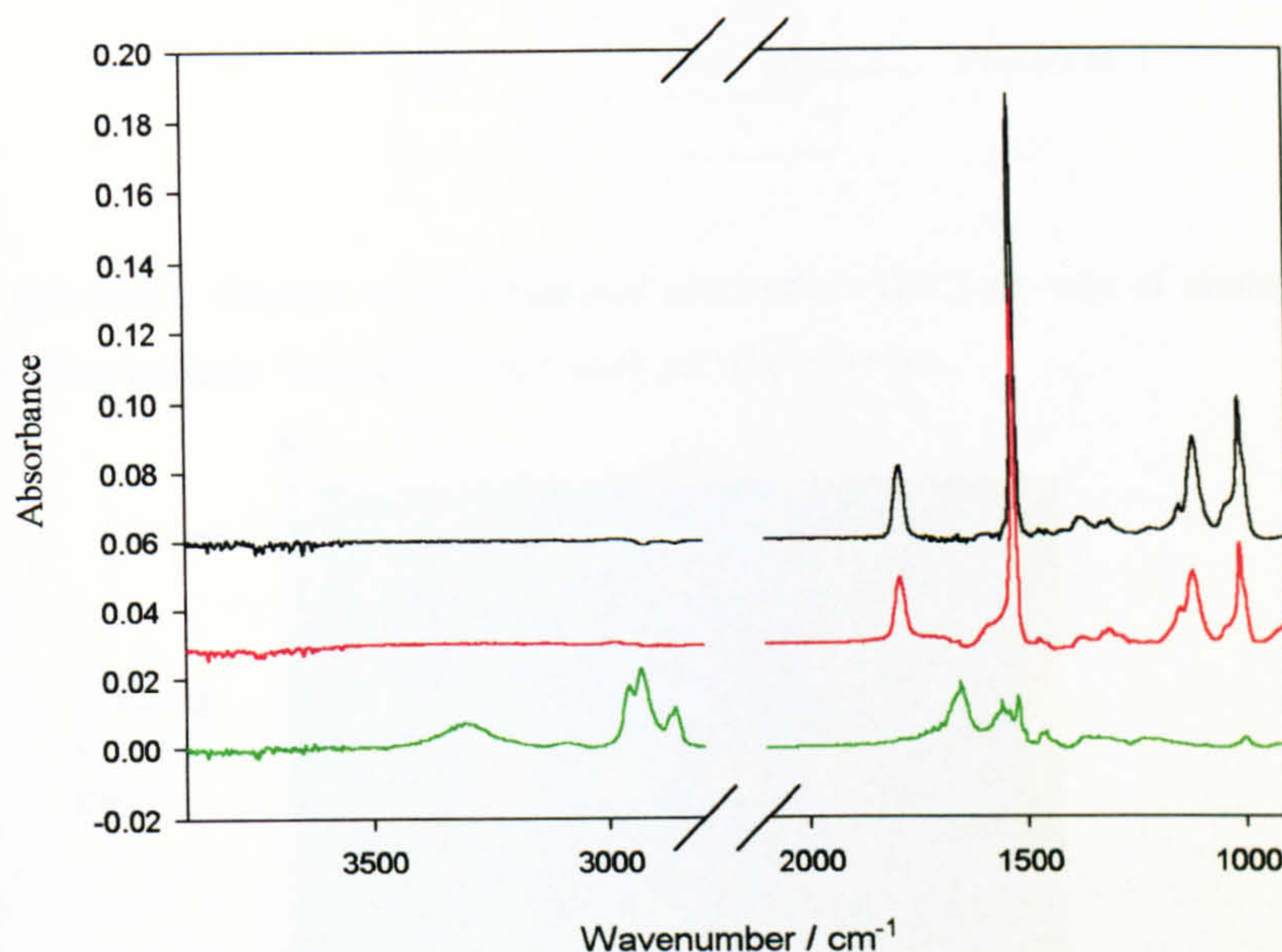


**Figure 5.6:** C (1s) XPS spectrum (TOA 90°) of poly(PFP) films after immersion in DMSO solution containing 0.5 mM hexylamine for 5 minutes; undoped polymer (red) and doped (at 0.77 V) polymer (black). The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

### 5.2.3 Selective Patterning of Ferrocene Ethylamine on Poly(PFP) Modified Electrodes

To demonstrate how the method described in Section 5.3.1 could be used for selective patterning on addressable electrodes on the same device poly(PFP) was deposited onto the fingers of interdigitated electrodes. By controlling the thickness of the electrodeposited film it is possible to prevent the growing polymer bridging neighbouring electrodes (this was confirmed by visual observation under a microscope and by checking with a conductivity meter).

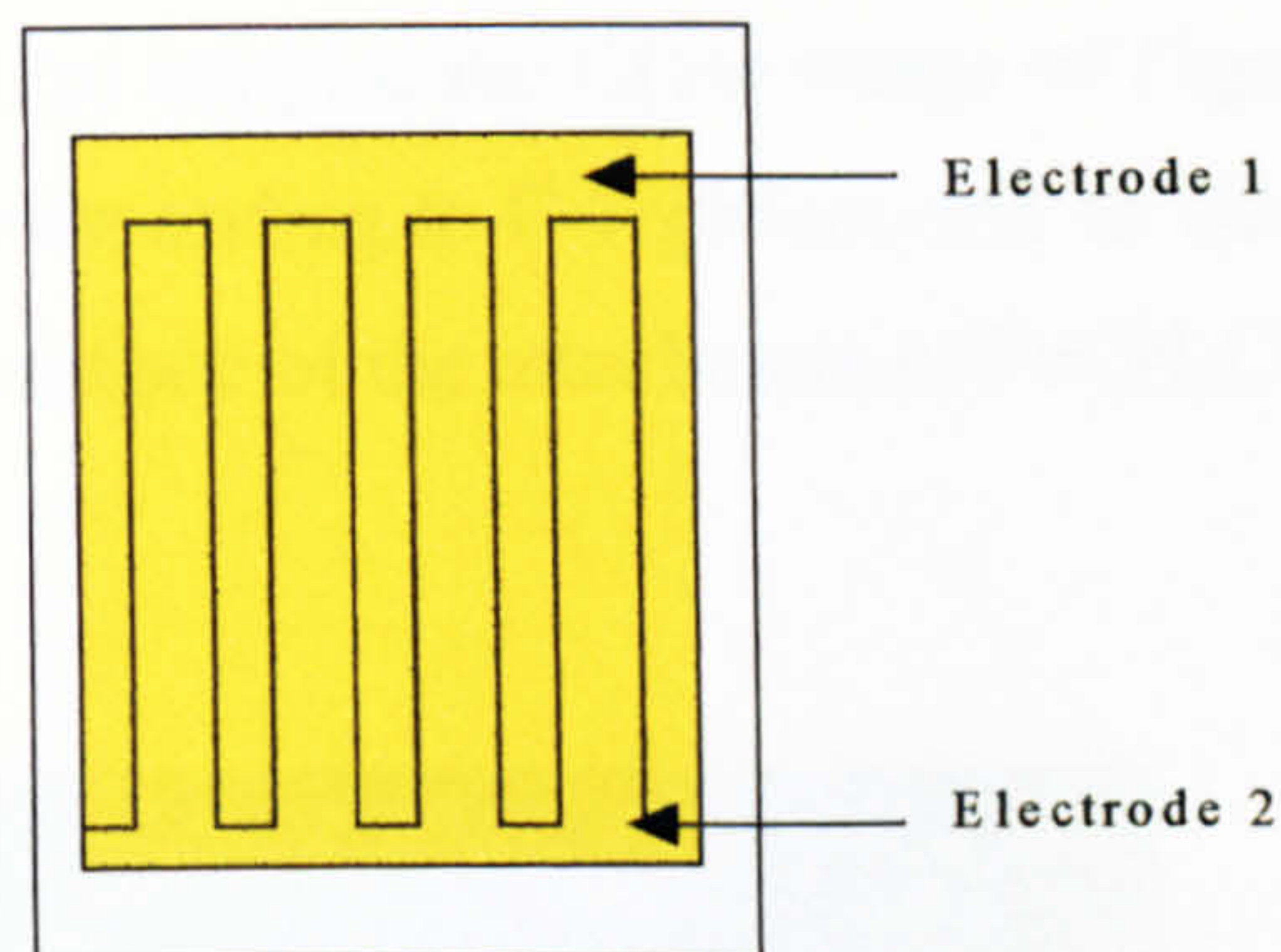




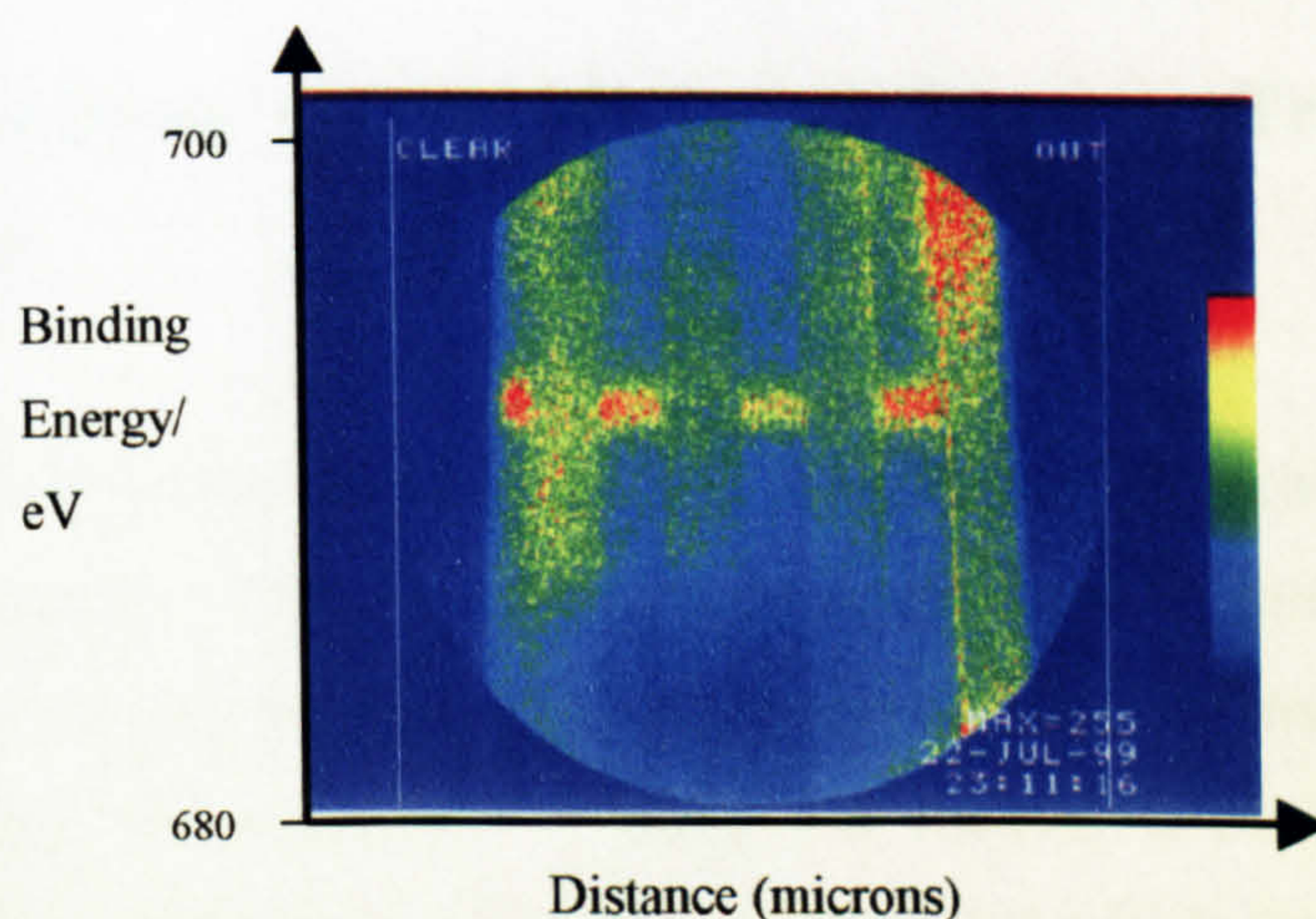
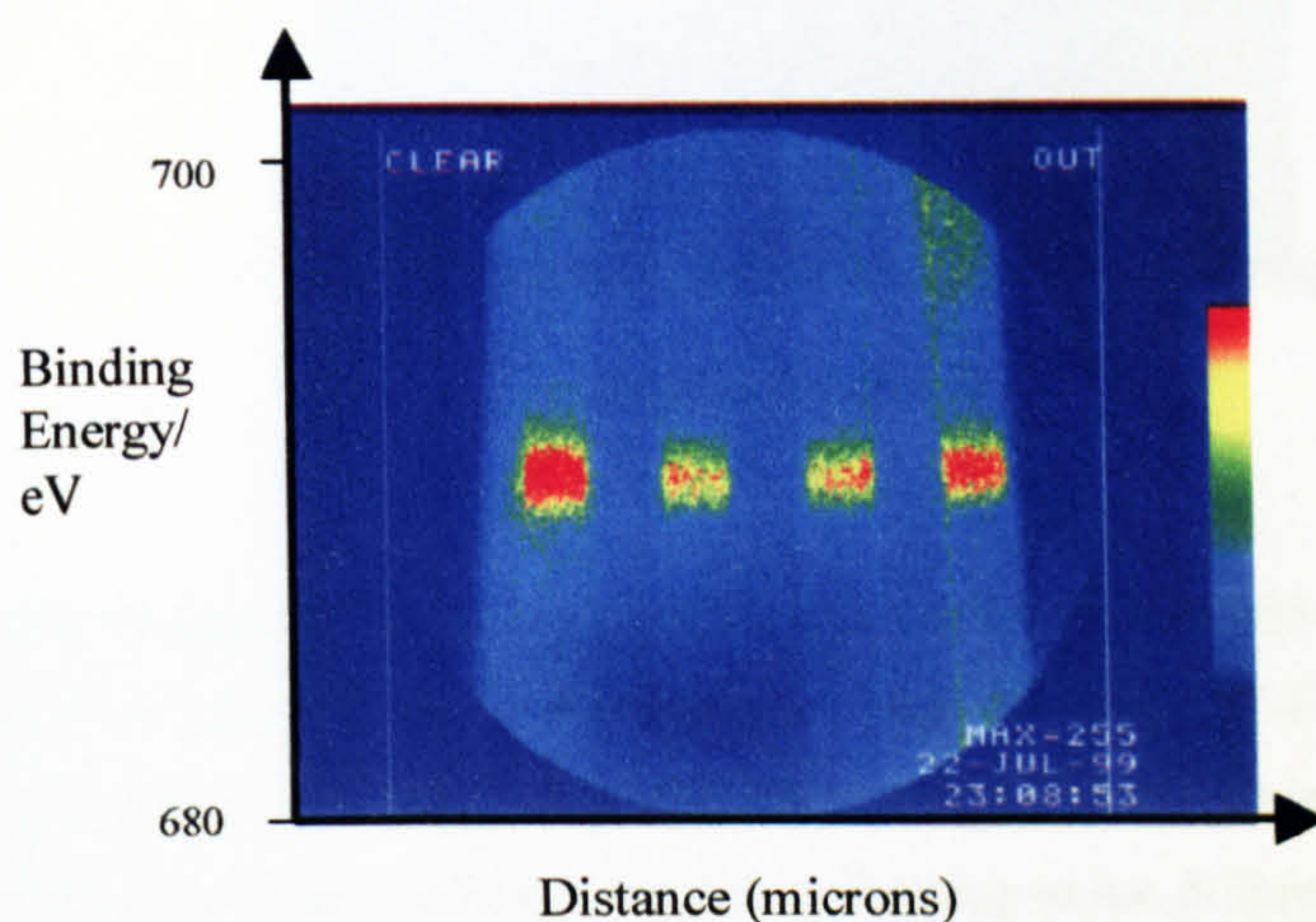
**Figure 5.7:** RAIRS absorbance spectra for poly(PFP) films unreacted and after immersion in 0.5 mM hexylamine and DMSO solution at specified electrochemical conditions: (a) unreacted poly(PFP) film (black), (b) oxidatively doped (at 0.77 V) poly(PFP) (red), (c) undoped poly(PFP) (green). The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

By performing XPS imaging experiments it was possible to demonstrate selective patterning of ferrocene ethylamine on one set of specific electrodes of a device. The F (1s) and Fe (2p) regions of the image are characteristic of the unreacted and reacted polymer respectively. It can be seen from Figure 5.9 that for the set of interdigitated electrodes (Figure 5.8) that FcCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub> has been immobilised there is a Fe (2p) signal on each alternate electrode. For the set of electrodes, which were doped, there was still an F (1s) signal apparent.





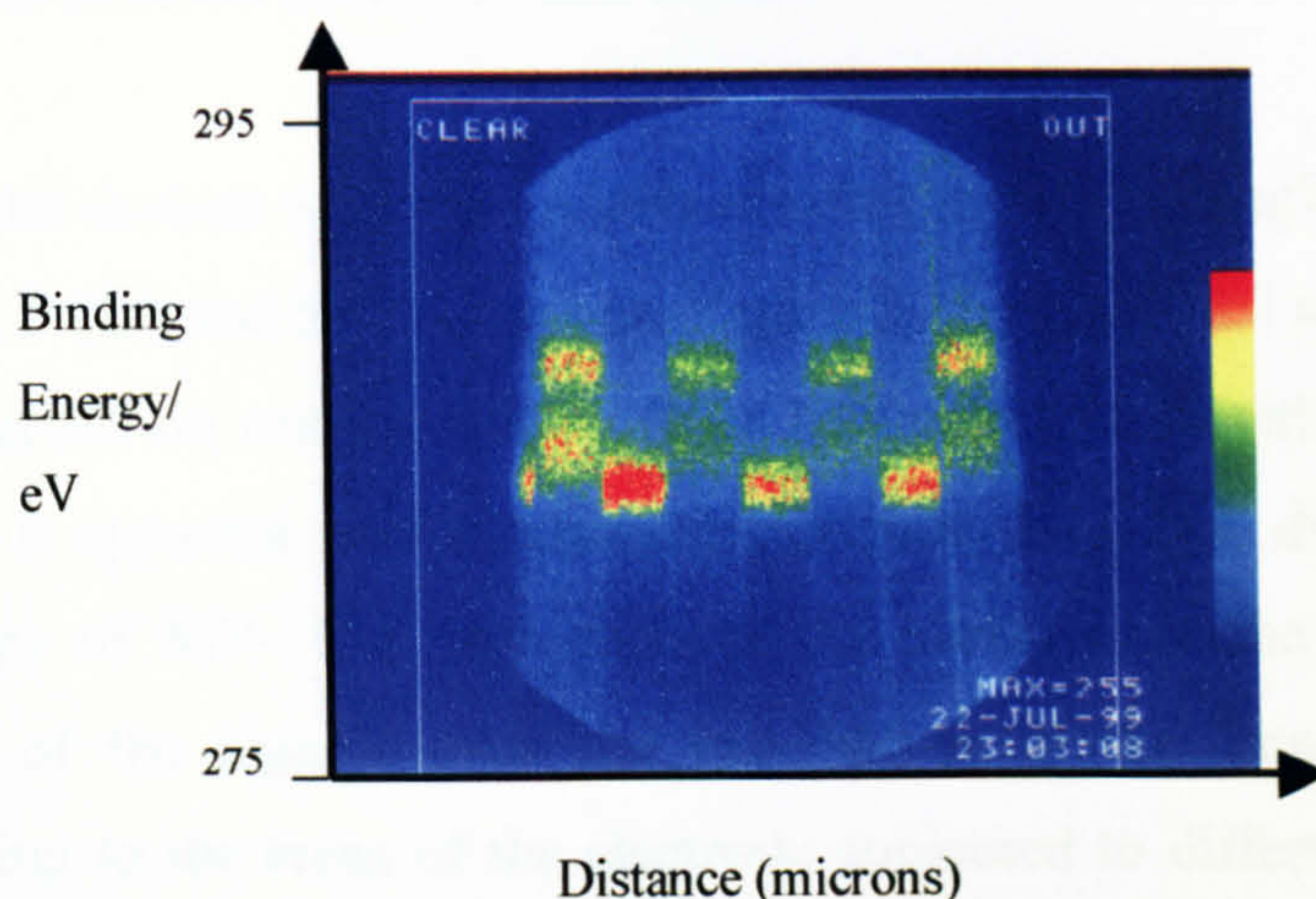
**Figure 5.8:** Schematic diagram of interdigitated electrodes. The both sets of electrodes are labelled and the black lines indicate the gap between each set of electrodes.



**Figure 5.9:** XPS Imaging of (a) F (1s) region and (b) Fe (2p) region of the interdigitated electrode device after being immersed in 1 mM ferrocene ethylamine and DMSO solution. Note that Fe (2p) signal is only present on the set of electrode, which were undoped and that F (1s) signal is still apparent from the doped polymeric electrodes. The colour coding on the left of the image indicates the colour relating to the different intensity of signal, where red is high intensity. Distance is 0-500  $\mu\text{M}$ .



Further to the F(1s) and Fe(2p) images, the C(1s) image of Figure 5.10 shows there has been a loss of signal corresponding to C-F groups and an increase in aromatic C-H and amide regions, characteristic of the attachment of  $\text{FcCH}_2\text{CH}_2\text{NH}_2$ .



**Figure 5.10:** XPS Imaging of C (1s) region of the interdigitated electrode device after being immersed in 1 mM ferrocene ethylamine and DMSO solution. Note the loss of C-F region and increase in C-H region for the polymer-modified electrode that was undoped while immersed in solution. The colour coding on the left of the image indicates the colour relating to the different intensity of signal, where red is high intensity.

#### 5.2.4 Patterning of Biological and Electron Transfer Motifs on Poly(PFP)

It would be of interest within the field of analytical biotechnology to show that a biological species, with a distinct spectroscopic probe, could be immobilised on poly(PFP), and accordingly the microenzyme MP-11 was used during these investigations. The advantage of using this enzyme is that it has a S (2p) label present (due to cystamine units in the amino chain) which therefore, makes it “easy” to detect. Due to the weaker signal of MP-11 modified electrodes (see Section 3.4.3.1), a large area two-digit electrode was fabricated (500  $\mu\text{M}$  wide) and was used to immobilise two nucleophilic species,  $\text{FcCH}_2\text{CH}_2\text{NH}_2$  and MP-11, on the different electrodes.

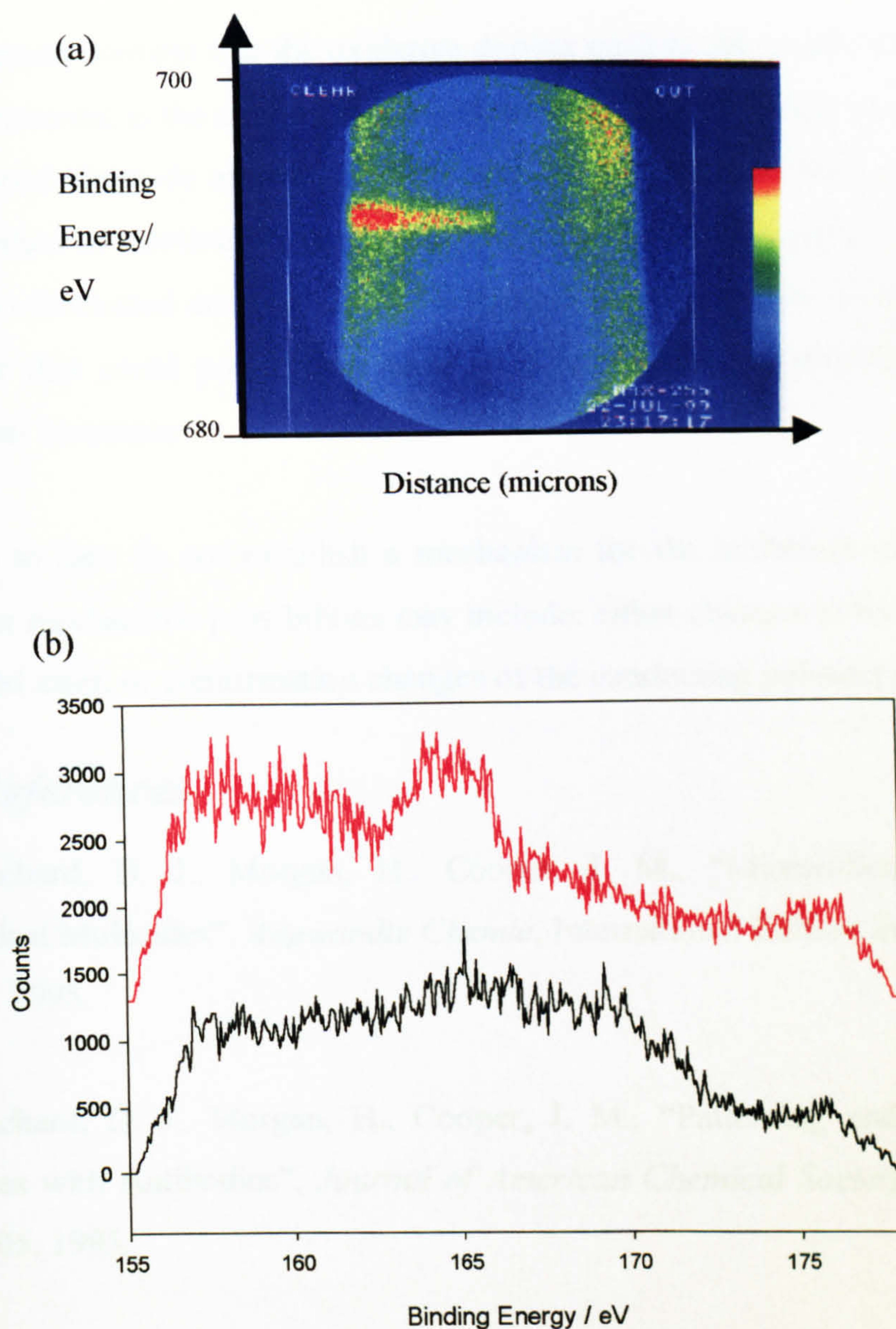


Since MP-11 immobilisation had been found to occur at a much slower rate than that of  $\text{FcCH}_2\text{CH}_2\text{NH}_2$ , the PFP substrate was first immersed in a ferrocene ethylamine solution, whilst one set of the electrode fingers was held in the oxidatively doped state. After reduction of the doped electrode the substrate was then immersed in a semi-aqueous solution of MP-11 and DMSO.

As before, the devices were then analysed using the XPS Imaging facility to measure the Fe (2p), F (1s) and S (2p) regions. Figure 5.11 shows that a strong Fe (2p) signal can be observed on one electrode. After further reaction with MP-11 solution a S (2p) signal is apparent on the adjacent electrode, however, due to the weak signal from S (2p) of MP-11 it was difficult to observe on the image obtained. An integration of the signals over the two halves of the image (Figure 5.10 (b)) corresponding to the areas of the electrode subjected to different electrode potential regimes shows that a S (2p) signal is apparent only on one electrode.

To investigate whether it was possible to pattern a second biological species on poly(PFP) modified interdigitated electrodes, experiments were carried out using cystamine (discussed further in Chapter 7). Initially the whole device was immersed in an electrolyte containing acetonitrile solution. One electrode was left undoped (0 V), whilst the second polymer modified electrode was oxidised (0.77 V) to allow doping to occur. The device was then placed in a 1 mM cystamine/DMSO solution for 15 minutes. After this period of time the device was placed in the cystamine free electrochemical solution and the oxidised polymer was then reduced to 0 V (results can be seen in Figure 7.18 in Chapter 7).





**Figure 5.10:** XPS image of (a) the Fe (2p) region and XPS spectra of one electrode and (b) the Fe(2p) (black) and S (2p) (red) region of the alternative electrode on the same device. The presence of Fe (2p) is apparent on the electrode that was undoped whilst immersed in ferrocene ethylamine and DMSO solution but not on the doped polymer. A weak S (2p) signal is apparent on the alternative electrode after the polymer was reduced and then immersed in a semi-aqueous DMSO solution of MP-11.



### 5.3 Summary

These results confirm that the oxidative doping inhibits the reaction of one electrode, while immersed in the amine solution. Moreover, the substitution reaction still occurs on a doped electrode after it has been undoped i.e. the inhibition is not permanent. This method of reactivity control could be useful for the patterning of various species on micro-fabricated arrays and reports the initial investigations of preparation of the polymer that could possibly be used as a matrix for the production of a multi-analytical biosensor.

Results to date do not establish a mechanism for the inhibition of these reactions, however mechanistic possibilities may include: either changes in bond strength of the activated ester; or conformation changes of the conducting polymer backbone

### 5.4 References

1. Pritchard, D. J., Morgan, H., Cooper, J. M., "Micron-Scale Patterning of Biological Molecules", *Angewandte Chemie*, International Edition in English, vol. 34, pp. 91, 1995.
2. Pritchard, D. J., Morgan, H., Cooper, J. M., "Patterning and Regeneration of Surfaces with Antibodies", *Journal of American Chemical Society*, vol. 67, no. 19, pp. 3605, 1995.
3. Morgan, H., Pritchard, D. J., Cooper, J. M., "Photo-patterning of sensor surfaces with biomolecular structures: characterisation using AFM and fluorescence microscopy", *Biosensors and Bioelectronics*, vol. 10, pp. 841, 1995.
4. Hadyoon, C. S., Glidle, A., Morris, D. G., Cooper, J. M., "Electrochemically controlled micropatterning of immobilised species on functionalised electrode interfaces", *Chemical Communication*, pp. 1683-1684, 1999.
5. Price, W. E., Too, C. O., Wallace, G. G., Zhou, D., "Development of membrane systems based on conducting polymers", *Synthetic Metals*, vol. 102, pp. 1338, 1999.

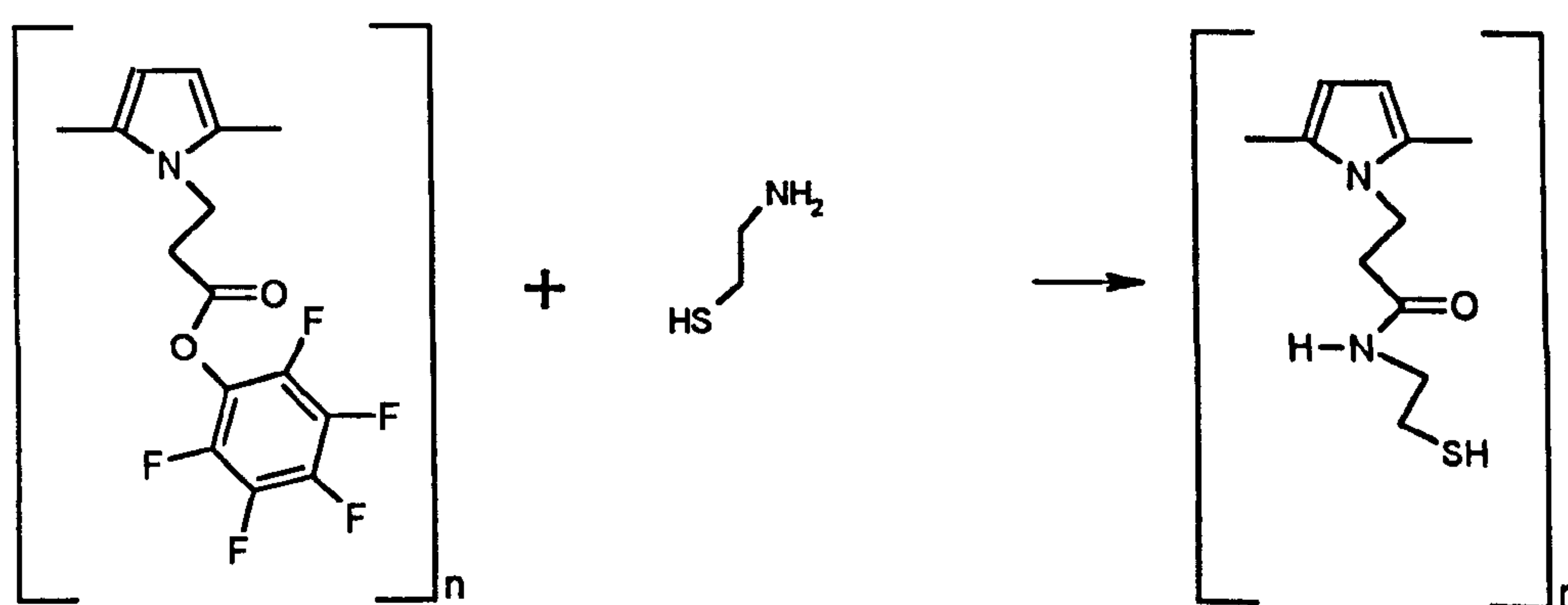


# Chapter 6

## *Reaction of Cystamine Modified Poly(PFP) Polymers*

### *6.0 Introduction*

In earlier chapters, the simple synthetic amines, ferrocene ethylamine and hexylamine, were used to illustrate aspects of the nucleophilic substitution reaction with the activated ester groups of poly(PFP). This chapter now investigates the reaction (and subsequent uses) of poly(PFP) with the bifunctional amine cystamine. As can be seen from the modification scheme of poly(PFP) below (Figure 6.1), the resulting polymer bears thiol groups.



**Figure 6.1:** Reaction of poly(PFP) with cystamine. The amine group of the cystamine react with the activated ester of the polymer to form an amide bond.



Research involving the incorporation of metals within polymer interfaces have been reported<sup>1-3</sup> and as a result of generating a polymer interface, with available thiol groups, the poly(PFP) electrode can be transformed into a template for the subsequent specific immobilisation of the desired molecules/ biomolecules through metal ion chelation or covalent bond forming reactions<sup>4-7</sup>. An example could possibly be the immobilisation of nucleoside bases, which have amine groups present, available to react with metal centre. Significantly a number of immobilisation schemes are available with a thiolated interface that employ chemistries complementary to the nucleophilic substitution of the parent PFP polymer (e.g. the reaction between cystamine and malimide or iodoacetyl groups (described in Chapter 6) and co-ordination complex formation described here).

To develop a multicomponent system that could ultimately be used in biosensor technology it was important to characterise the surface modifications occurring at each stage of the process. The opening of this chapter concentrates on the nucleophilic reaction of cystamine with the homo and copolymer systems. The modified homopolymer template was then used for subsequent reaction with a platinum complex. XPS was used to investigate the surface co-ordination of the platinum complex, whereas, QCM was used to establish the incorporation of the material into the bulk of the polymer membrane. Preliminary investigations were also performed to determine whether further immobilisation could occur around the Pt template via the formation of co-ordination bonds around the chelated Pt centre and various miscellaneous species.

The experimental strategy employed to fabricate a multicomponent system is discussed in greater detail in later sections. However, these (successful) investigations were built on the electrochemical control technique described in Chapter 5 to control the cystamine immobilisation reaction. Thus, a suitable sequence of controlled cystamine reaction followed by further specific immobilisations offer the opportunity for multi-patterning of various species on individually addressable electrodes.



## ***6.1 Materials***

**Solvents:** DMSO (99+%, anhydrous) and MeCN (99.5+%, ACS reagent) were obtained from Aldrich.

**PFP, TEAP and Pyrrole:** were prepared as described in Chapter 2 Section 2.1.

**Cystamine** obtained from Sigma

**K<sub>2</sub>PtCl<sub>4</sub>** (Potassium tetrachloroplatinate(II), 99.99%) was obtained from Aldrich.

**Ligands:** Ethanolamine (2-aminoethanol, 99+%), iodobenzylamine (3-iodobenzylamine, 96%) and fluorothiophenol (4-fluorobenzenethiol, 99%) were obtained from Aldrich. Ferrocene ethylamine was prepared as described in Chapter 4 section 4.1.

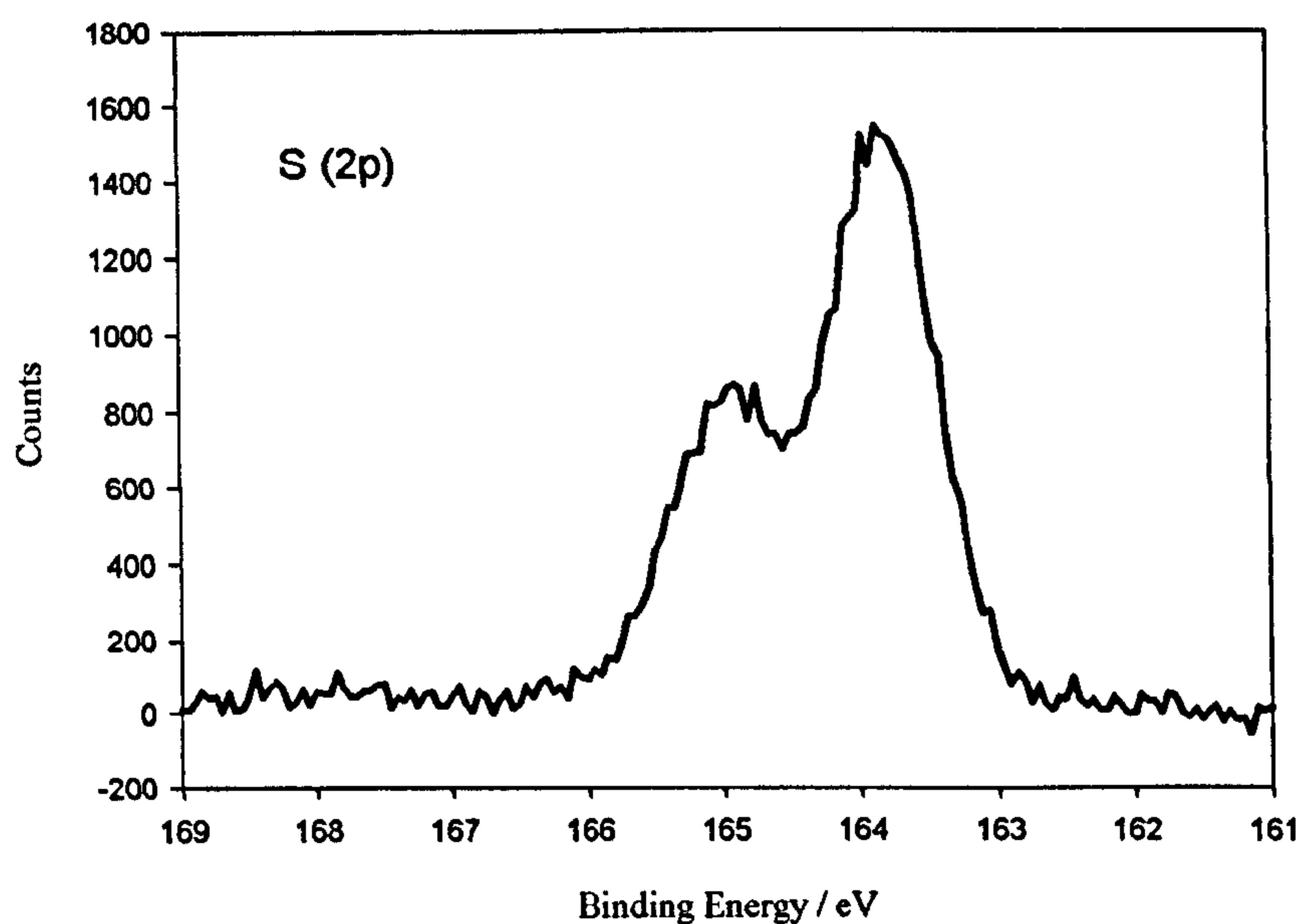
## ***6.2 Results and Discussion***

### **6.2.1 Production of Cystamine Modified Polymer**

#### **6.2.1.1 Characterising the Bonding in Homopolymer Reaction with Cystamine**

Due to the low reactivity of poly(PFP) electrodes in aqueous solutions (Section 6.2.1.2), cystamine modification was accomplished by immersion in cystamine/dimethylsulphoxide (DMSO) solutions. The resultant polymer films were analysed using XPS and RAIRS. The XPS spectrum collected for the poly(PFP) after reaction with cystamine has the appearance of the S (2p) doublet and thus indicated the presence of cystamine on the surface layers on the polymer-modified electrode, as seen in Figure 6.2. It was shown from XPS analysis that the ratio of S:N:O is 1:2:1 as would be expected if poly(PFP) has completely reacted with cystamine.

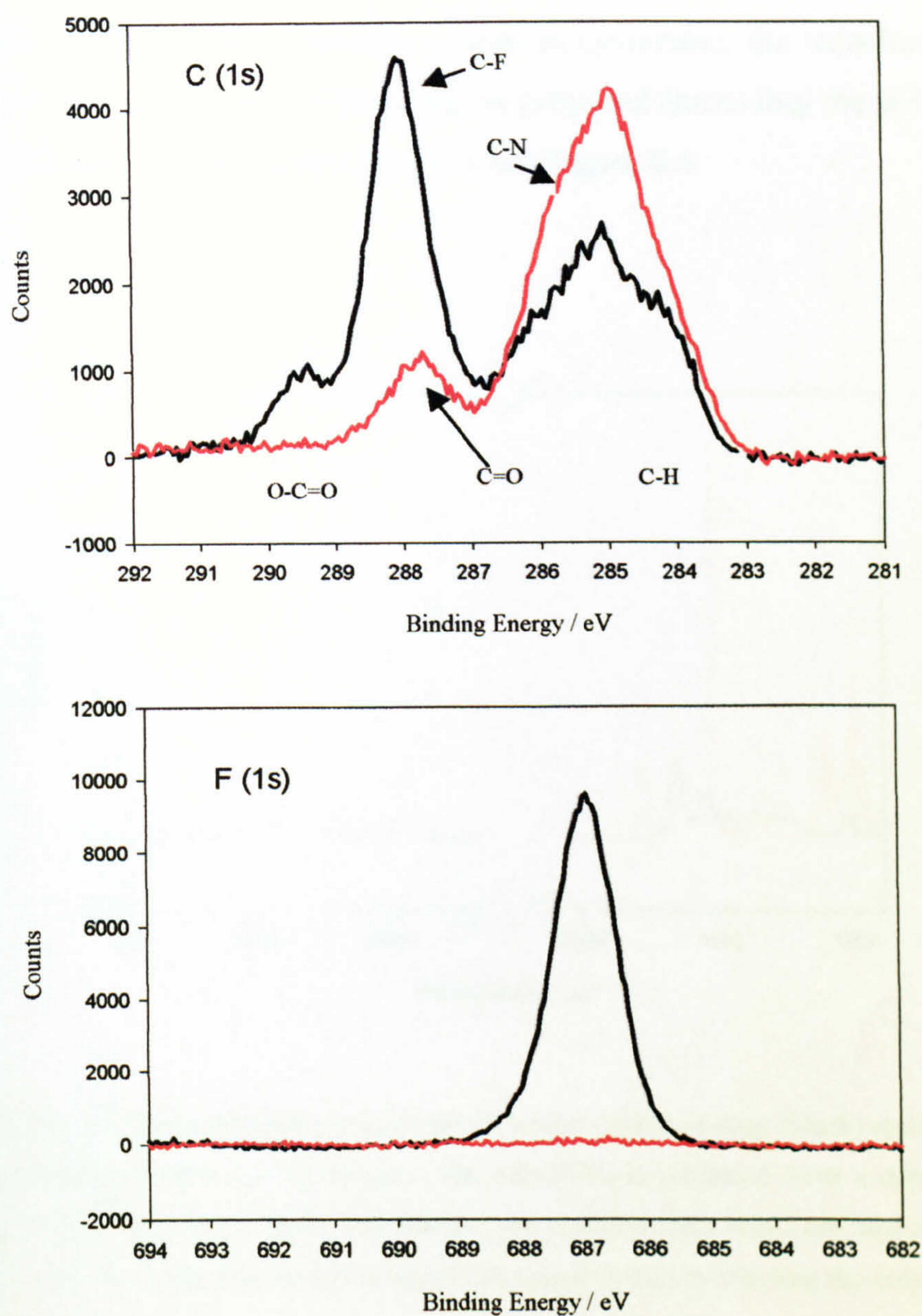




**Figure 6.2:** S (2p) XPS spectrum (TOA 90 degrees) of modified poly(PFP) film (on 1cm<sup>2</sup> gold electrode) after reaction with 1 mM cystamine and DMSO solution for 15 minutes. The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

The C (1s) spectrum (Figure 6.3) shows there is an absence of C-F groups at 288 eV and the lack of fluorine species is confirmed by the F (1s) spectrum (Figure 6.3). The presence of the C=O peak at 287.5 eV and the C-N shoulder at approximately 286 eV in the C (1s) spectrum are characteristic of amide formation.

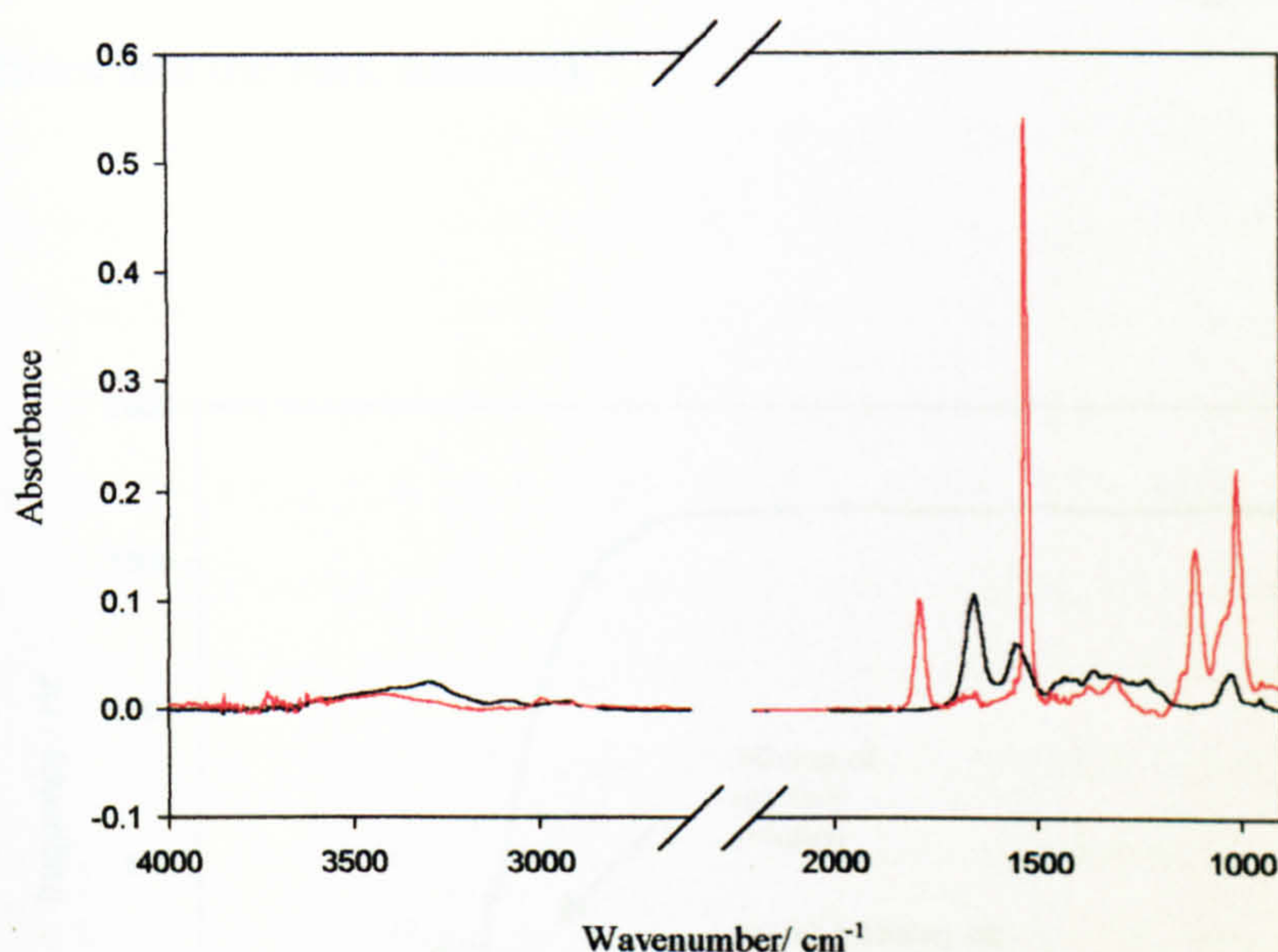




**Figure 6.3:** C (1s) and F (1s) XPS spectra (TOA 90 degrees) of cystamine modified poly(PFP) film (red) and unreacted poly(PFP) film (black). The poly(PFP) is produced by scanning the potential to 1.02 V in a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used. The cystamine modified poly(PFP) was prepared by reacting the polymer with 1 mM cystamine/ DMSO solution.



RAIRS was used to determine whether the reaction of the activated ester with cystamine involved the formation of an amide or thioester bond as the thiol group of cystamine can also act as a nucleophile. As would be expected from the relative nucleophilicities<sup>8</sup> of the two terminal groups in cystamine, the substitution reaction leads to the formation of an amide group, as proposed (providing the pH was not too alkaline, as this will generate  $RS^-$  species), see Figure 6.4.



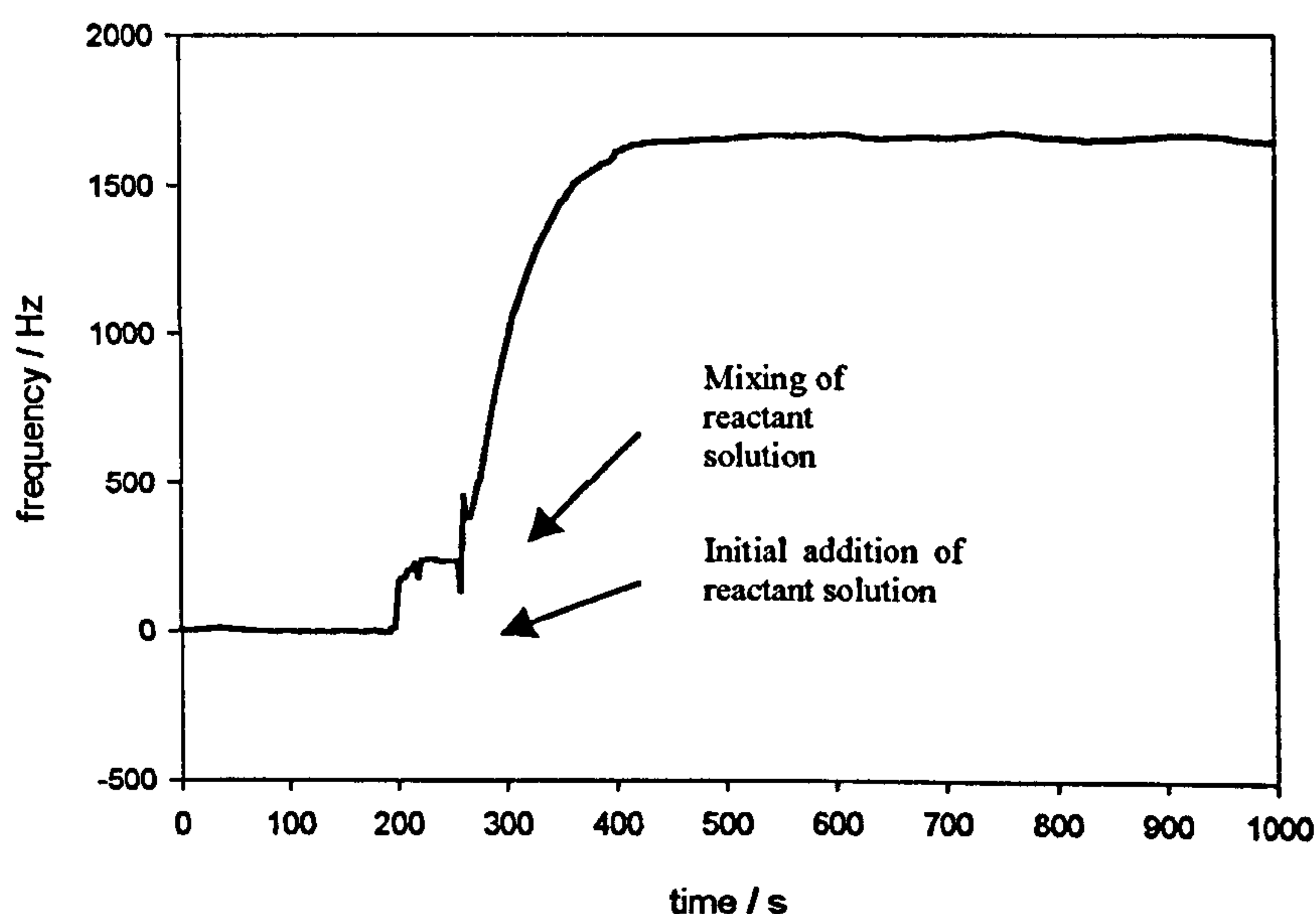
**Figure 6.4:** RAIRS spectra of unreacted poly(PFP) before (red) and after (black) reaction with 1 mM cystamine/ DMSO solution for 30 minutes. The poly(PFP) is produced from a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used. The cystamine modified poly(PFP) was prepared by reacting the polymer with 1 mM cystamine/ DMSO solution.

#### 6.2.1.2 Characterising the Kinetics of the Cystamine–Homo Poly(PFP) Reaction

As previously discussed XPS only investigates the polymer-solution interface so the EQCM method was used to determine both when a polymer reaction has taken place and to determine the length of time for complete substitution of the pentafluorophenyl group with cystamine. The changes in the crystal resonance frequency are linearly related to mass changes in the polymer layer. Figure 6.5 shows



the time course of the QCM frequency changes when poly(PFP) coated quartz crystal electrode was immersed in 1mM cystamine and DMSO solution. From reported work it was shown that there was an absence of frequency changes i.e. no reaction when the film was immersed in an aqueous cystamine solution due to the hydrophobicity of the poly(PFP) film<sup>4</sup>. In the case of the film being placed in a cystamine/ DMSO solution a large frequency increase was measured. The frequency increase occurred due to the fact that the leaving pentafluorophenyl group has a larger mass than the entering cystamine species thus a nucleophilic substitution by cystamine leads to an overall reduction in films mass (assuming the fluorophenol group diffuses into the bulk solution).



**Figure 6.5:** QCM frequency change (mass decreases) for a poly(PFP) coated electrode immersed in a 1mM solution of cystamine and DMSO. The poly(PFP) is produced from a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 0.229 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used.

For this reaction with cystamine and poly(PFP) the overall change in mass of an unsolvated polymer film after complete reaction would correspond to a loss of 35% of the original polymer mass. The measured frequency change on drying was approximately 1.75 kHz, indicating that the reaction has gone to completion (a dry



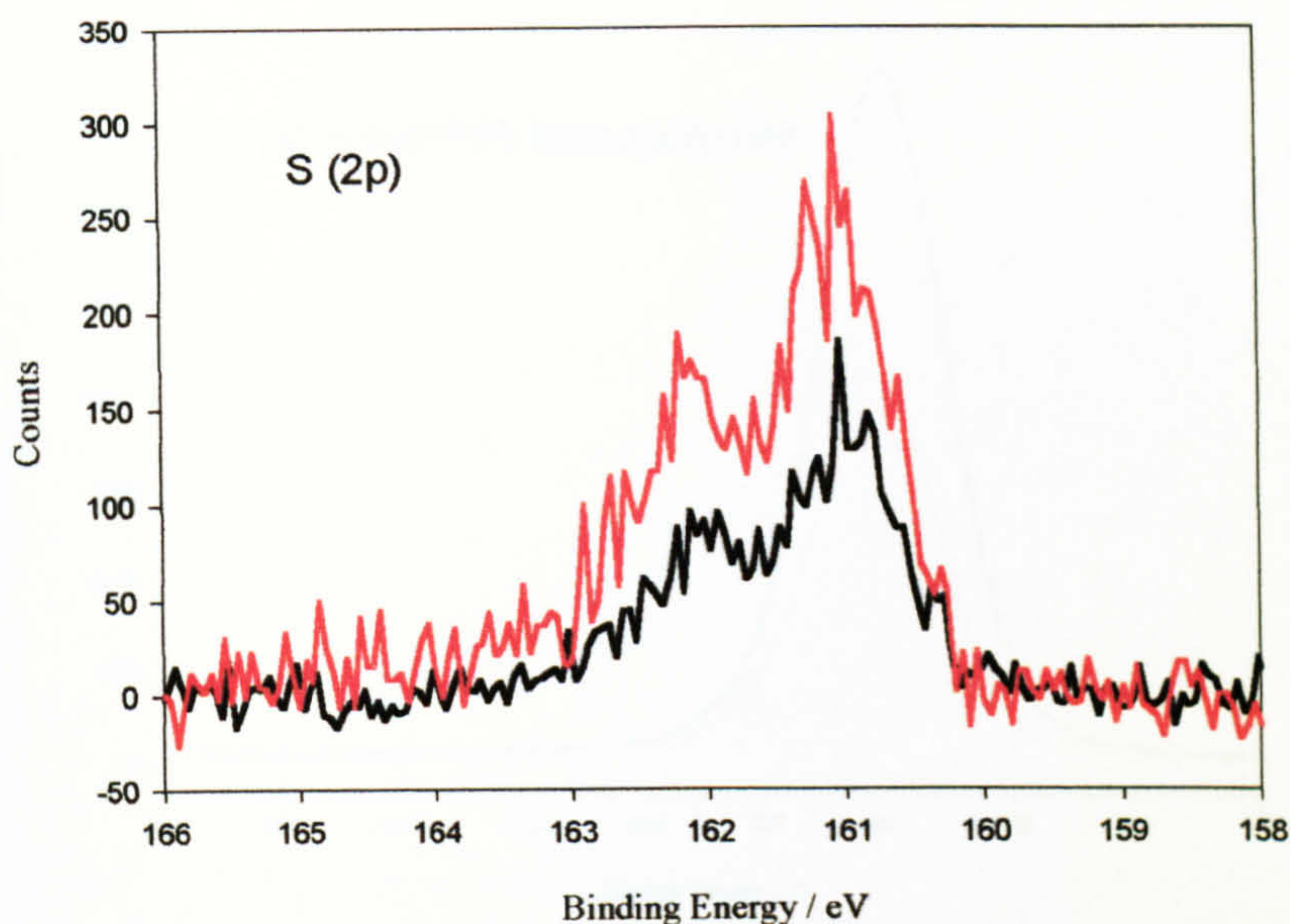
mass corresponding to a frequency difference of 1.729 kHz for the film used). Further, the similarity with solvated frequency measurements suggests that little extra solvent incorporated following substitution with cystamine.

#### **6.2.1.2 Copolymer Reaction with Aqueous Cystamine**

Whilst the solubility of cystamine in organic solvents such as DMSO is sufficiently high to allow the nucleophilic reaction with the homopolymer, poly(PFP), the smallness and solubility in water of this nucleophile also suggests its use as a probe of the reaction of Py/PFP copolymers in aqueous solution. Thus, the reactions of copolymer films, prepared as described in Chapter 2, with cystamine (aqueous) were investigated. As before measurements of the F (1s) XPS signal can be readily used to determine whether a reaction at the Py/PFP copolymer interface has occurred. XPS analysis of the F (1s) and S (2p) signal from a series of Py/PFP copolymer films immersed in an aqueous solution of cystamine was carried out. The results showed that the amount of cystamine immobilised at the polymer-solution interface gradually increased by 50% on changing the polymer composition from the homopolymer to a copolymer formed from a Py/PFP solution in which the solution mole fraction of PFP was 0.85. This is illustrated by the S (2p) XPS spectra of Figure 6.6.

Using the spectral deconvolution methods described in Chapter 4, it can be estimated that the 85:15 copolymer contained approximately 75% fewer potential surface immobilisation sites (the activated ester sites) than the homopolymer. Thus the difference in extent of reaction estimated by comparing the number of PFP groups remaining. Figure 6.7 shows that for the homopolymer, poly(PFP), approximately 45% of the surface PFP units do not react. In contrast, for the poly(Py/PFP) copolymer film (made from 0.85 solution mole fraction) greater than 90% of the groups react. The absolute number of F (1s) counts lost from the homopolymer is greater than in the copolymer and the amount of S (2p) counts is much smaller than expected, therefore, significant hydrolysis of the homopolymer has occurred.

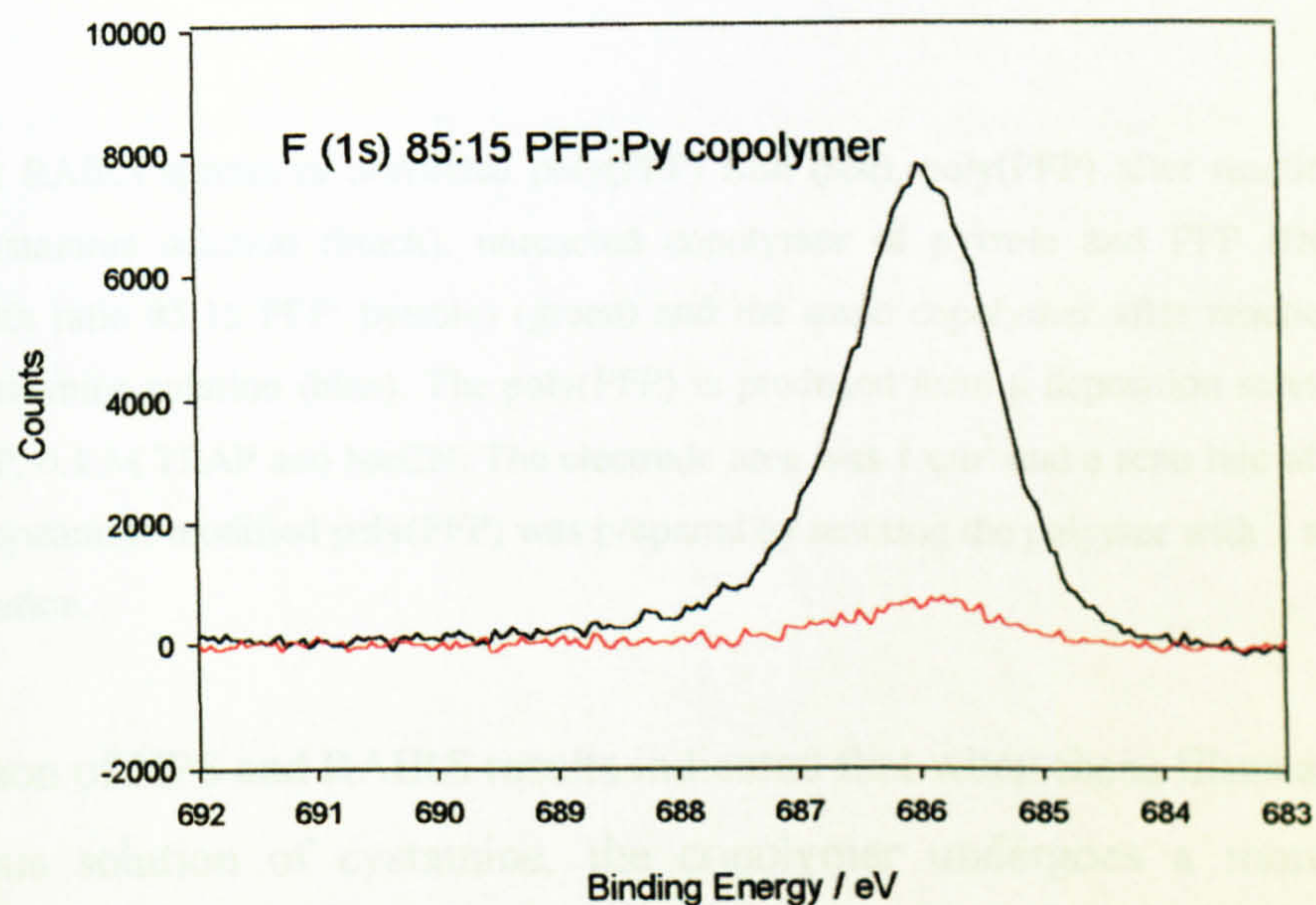
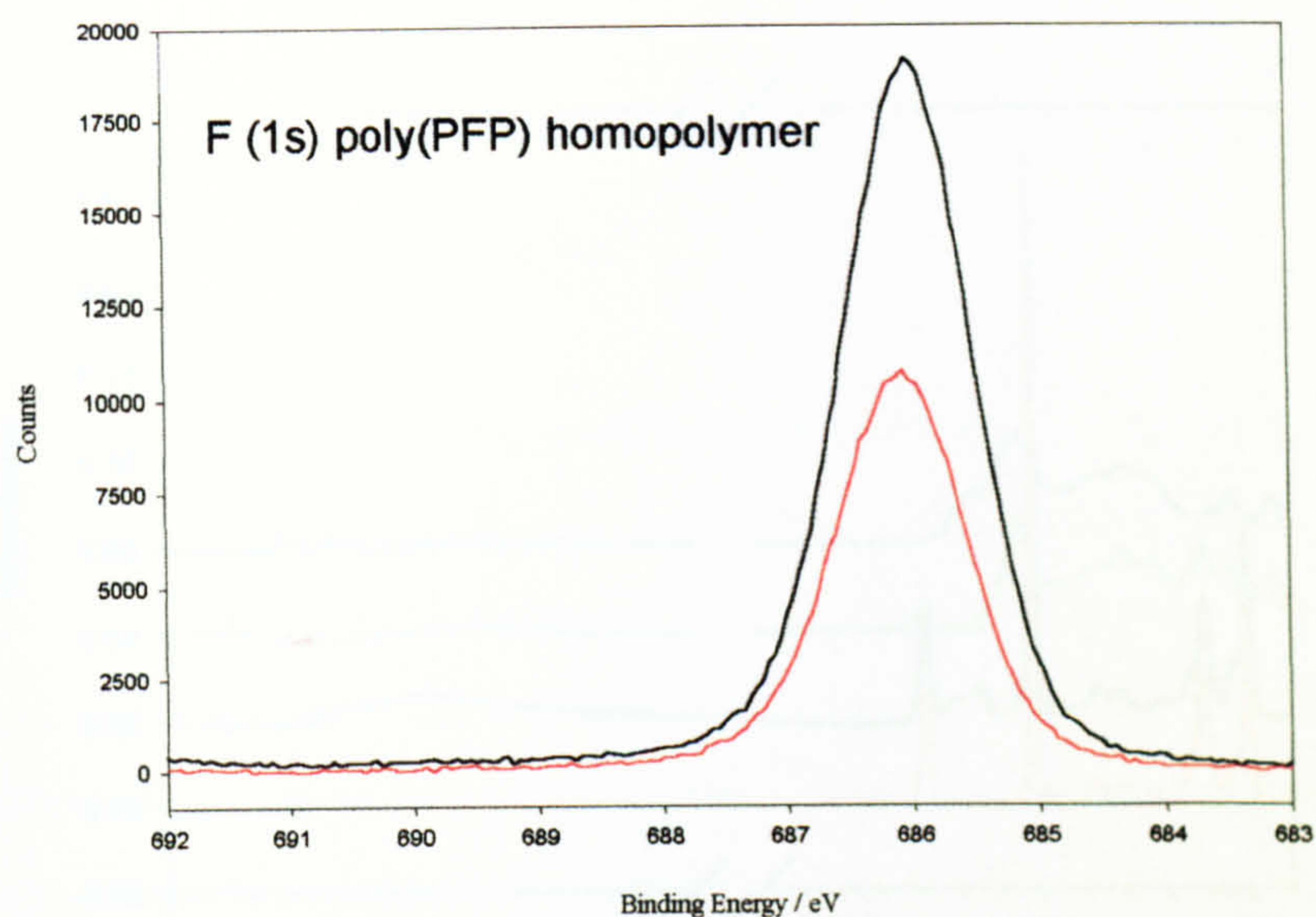




**Figure 6.6:** S (2p) XPS spectra (TOA 90 degrees) of (a) homopolymer (black line) and (b) 85:15 copolymer (red line) after reaction with a 1 mM aqueous cystamine solution. The homopolymer modified electrode (area 1 cm<sup>2</sup>) was prepared from a deposition solution containing 20 mM PFP and the copolymer modified electrode (area 1 cm<sup>2</sup>) from solution containing a ratio of 85:15 PFP: Pyrrole (both solution containing 0.1 M TEAP and MeCN and using a scan rate of 20 mVs<sup>-1</sup>).

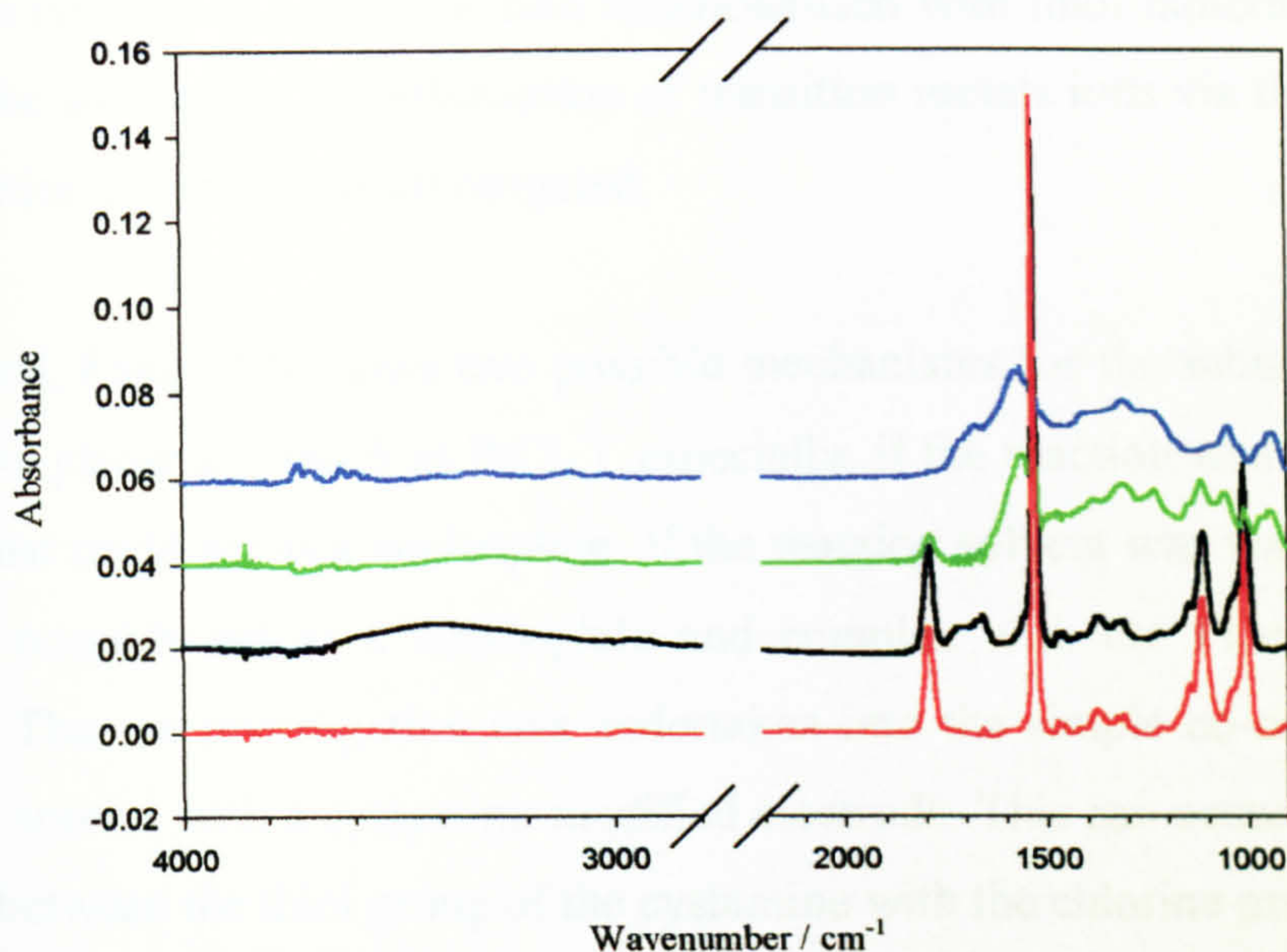
RAIRS measurements of the bulk of the polymers showed that, for the homopolymer, the signal from the activated ester group (at 1790 cm<sup>-1</sup>) decreased slightly, to 90% of that found in the unreacted polymer. In contrast, for copolymers, this signal decreased to a greater extent, and for the 0.85 mole fraction poly(PFP/Py) copolymer it was less than 10% of the unreacted value. In addition, along with the decrease in activated ester signal, an increasing band at 1670 cm<sup>-1</sup> was observed, which is characteristic of an amide group seen in Figure 6.8. The presence of the peak at 1720 cm<sup>-1</sup> in the black and as a shoulder in the blue spectrum is due to the presence of COOH peak as a consequence of hydrolysis occurring on the surface of the homopolymer and within the copolymer.





**Figure 6.7:** F (1s) XPS spectra (TOA 90 degrees) of homopolymer and 85:15 copolymer before (black) and after (red) reaction with aqueous cystamine solution. The homopolymer modified electrode (area  $1 \text{ cm}^2$ ) was prepared from a deposition solution containing 20 mM PFP and the copolymer modified electrode (area  $1 \text{ cm}^2$ ) from solution containing a ratio of 85:15 PFP: Pyrrole (both solution containing 0.1 M TEAP and MeCN and using a scan rate of  $20 \text{ mVs}^{-1}$ ).





**Figure 6.8:** RAIRS spectra of unreacted poly(PFP) film (red), poly(PFP) after reaction with 1 mM aqueous cystamine solution (black), unreacted copolymer of pyrrole and PFP (from deposition solution with ratio 85:15 PFP: pyrrole) (green) and the same copolymer after reaction with 1 mM aqueous cystamine solution (blue). The poly(PFP) is produced from a deposition solution containing 20 mM PFP, 0.1 M TEAP and MeCN. The electrode area was 1 cm<sup>2</sup> and a scan rate of 20 mVs<sup>-1</sup> was used. The cystamine modified poly(PFP) was prepared by reacting the polymer with 1 mM cystamine/DMSO solution.

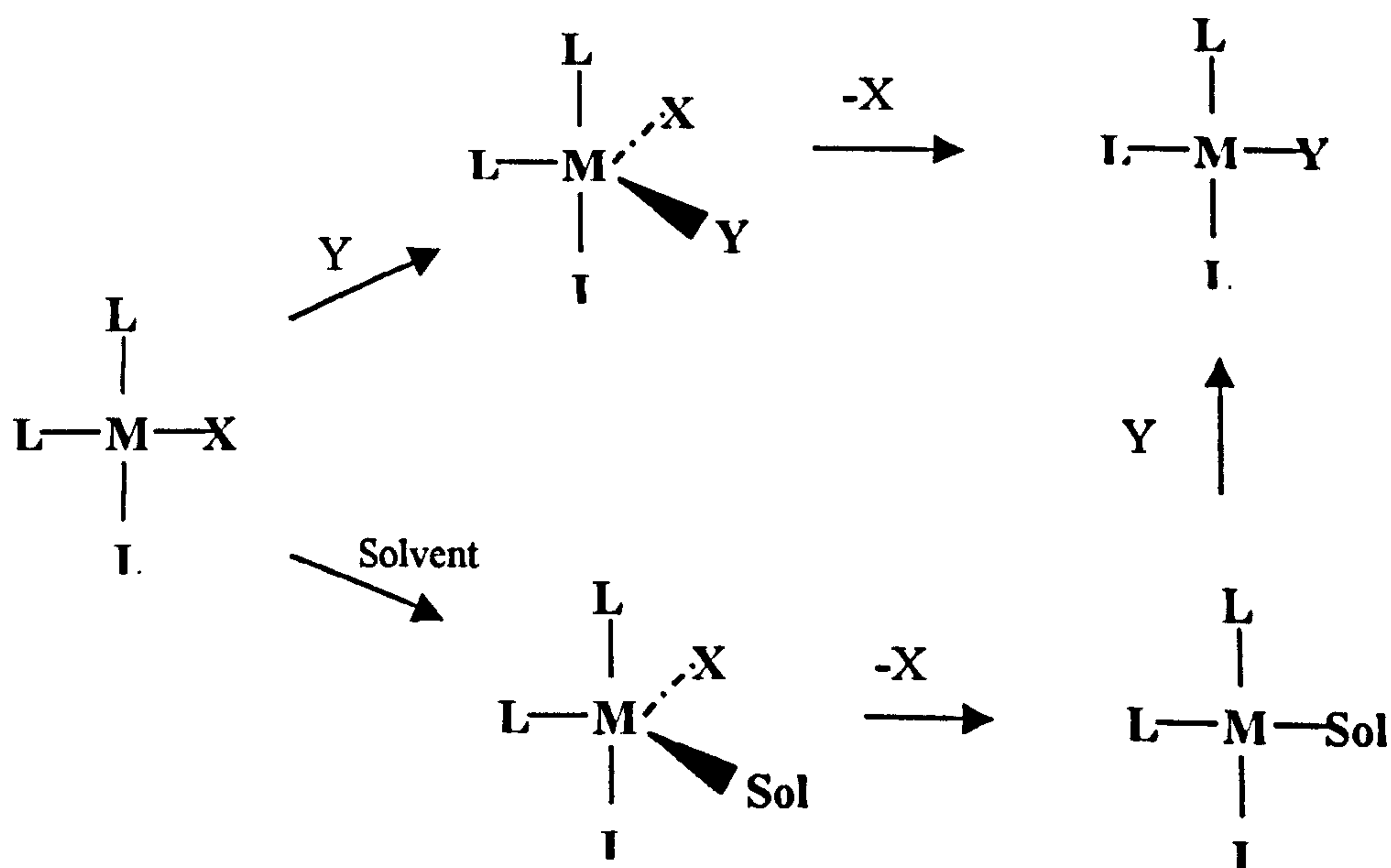
Comparison of XPS and RAIRS results indicated that when these films are immersed in aqueous solution of cystamine, the copolymer undergoes a more significant reaction throughout the matrix, whereas the reaction of the homopolymer is smaller and confined to the solution interfacial region. This was the result of the hydrophobic nature of the poly(PFP) film, making it difficult for aqueous based solutions to penetrate the bulk of the polymer matrix. Thus, for improved immobilisation of aqueous solvated biomolecules using this polymer system it would be desirable to use a copolymer system, which would increase the hydrophilicity of the bulk of the polymer or, additionally, semi-aqueous solutions (e.g. DMSO with small aqueous content) could also be employed.



## 6.2.2 Production of Cystamine-Platinum Template

As described in the previous section, the modification of poly(PFP) films with cystamine resulted in a polymer film functionalised with thiol moieties. Building up on this, the subsequent immobilisation of transition metals ions via the formation of co-ordination complex was investigated.

To this end, Figure 6.9 shows two possible mechanisms for the substitution reaction at a square planar site (such as  $\text{PtCl}_4^-$ ), especially, if the reaction was carried out in a solvent that could act as a nucleophile. If the reaction solvent was water, this species can also possibly act as a nucleophile and complex with the Pt centre in a side reaction. Thus an investigation was undertaken into the simple co-ordination of the platinum species with a cystamine-modified electrode. This can occur by substitution reaction between the thiol group of the cystamine with the chlorine present within the square planar complex. This reaction is thermodynamically feasible due to the fact that the platinum centre is a 'soft' metal ion and, therefore, can co-ordinate to soft donor species, such as the sulphur of the thiol from cystamine. In addition, the remaining ligands attached to the platinum centre, can be used for further immobilisation.



**Figure 6.9:** Scheme of the basic substitution reaction of a square planar site, where M=metal centre, L=leaving group and Y=reactive species.<sup>9</sup>



In addition to probing of the reaction of cystamine modified polymers with the  $\text{PtCl}_4^-$  complex, the selective immobilisation of the platinum complex is investigated via the electrode potential controlled reaction of cystamine with poly(PFP).

#### 6.2.2.1 Cystamine Modified Polymer Reaction with Platinum

For XPS measurements  $1\text{cm}^2$  cystamine modified poly(PFP) electrodes were prepared as described in a previous section. This electrode was immersed in a 10 mM  $\text{K}_2\text{PtCl}_4$  aqueous solution for 90 minutes. After reaction the sample was thoroughly washed and dried using  $\text{N}_2$  gas. The sample was analysed using the typical XPS conditions. The survey-scan spectrum and high resolution spectra of Pt (4f), Cl (1s), N (1s), O (1s) and S (2p) regions were collected. As discussed below, these XPS measurements were used to confirm that the proposed chelation and not physisorption of Pt to cystamine has occurred. Firstly, the presence of platinum is confirmed by the presence of a 4f doublet (Figure 6.10).

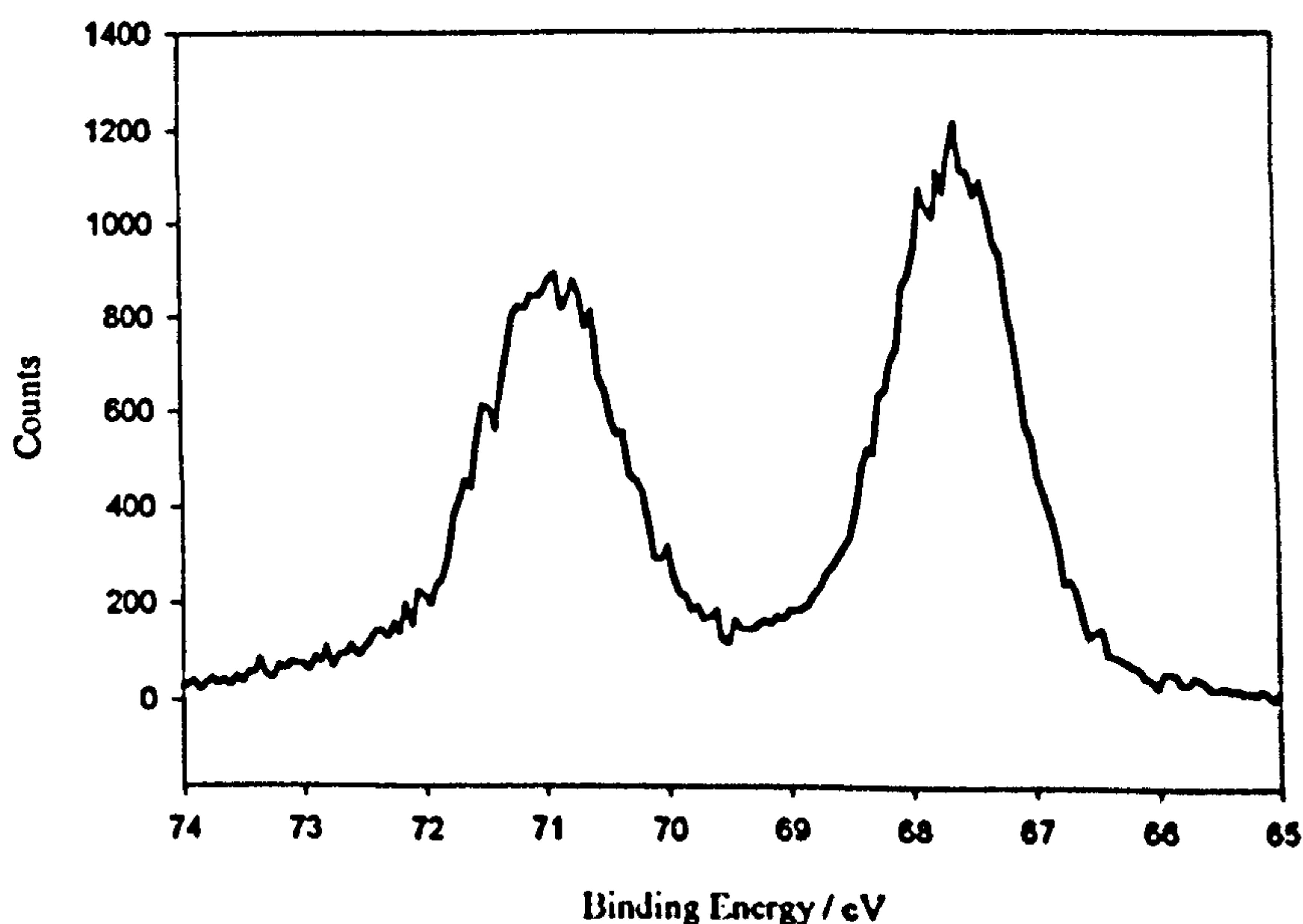
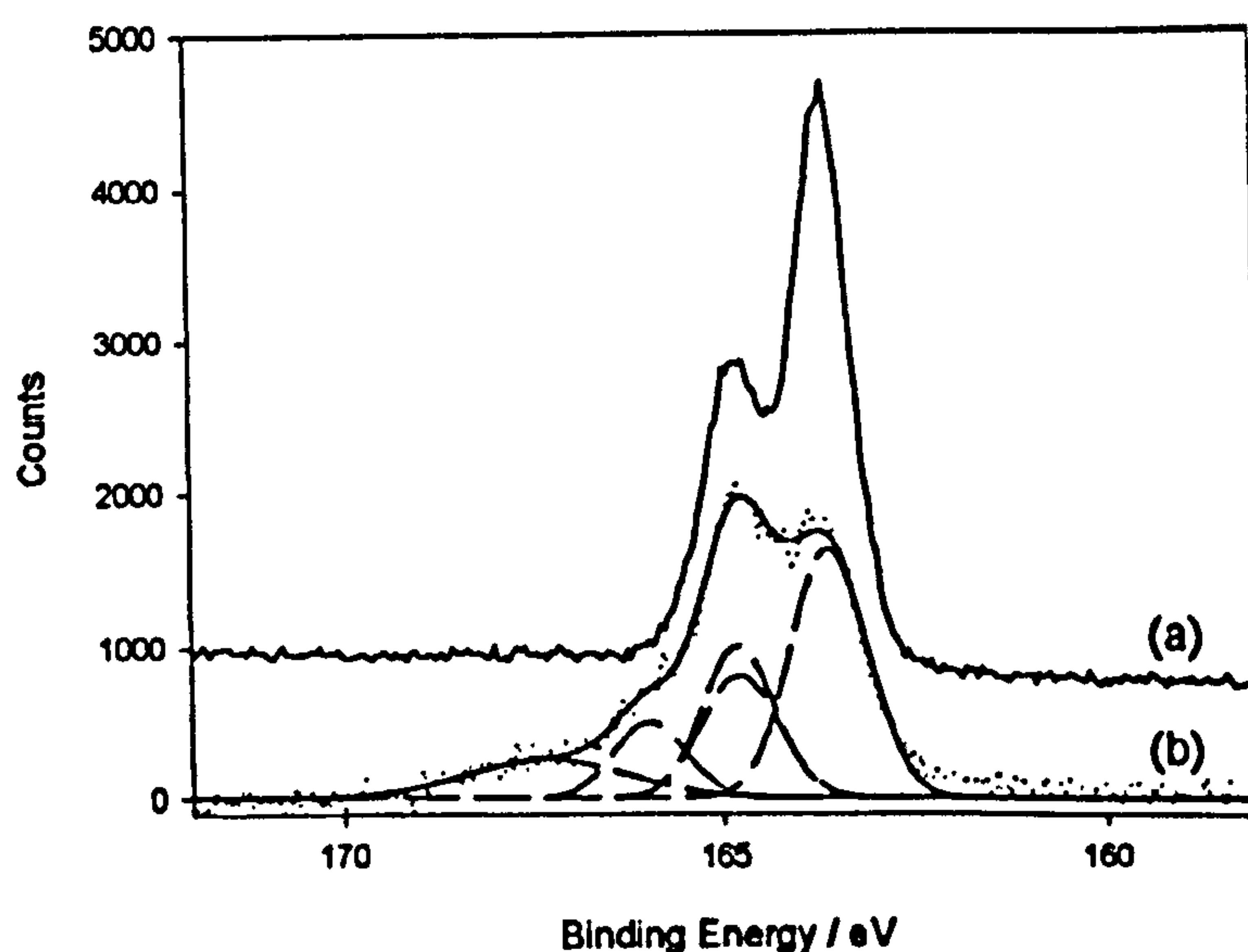


Figure 6.10: Pt (4f) XPS spectra (TOA 90 degrees) of cystamine modified poly(PFP) film after reaction with 10 mM aqueous  $\text{K}_2\text{PtCl}_4$  solution. The cystamine modified poly(PFP) film was produced by reacting the homopolymer film with 1 mM cystamine and DMSO.



The presence of two S (2p) doublets in the XPS spectrum of Figure 6.11 are due to sulphur centres that are either co-ordinated (165 eV) to Pt or remains uncoordinated (164 eV) by comparison with S (2p) energy from the unreacted film. This indicated that maximal Pt loading of the polymer has not been achieved. Deconvolution of the doublets show that 35% of the sulphur species was strongly coordinated to the Pt centre, with the remainder either being weakly coordinated or uncoordinated to the Pt centre (Figure 6.10). Importantly quantification of the Cl (2p) peak indicated that approximately one chlorine atom remains attached to the platinum centre and was, therefore, available for further substitution.



**Figure 6.11:** S (2p) XPS spectra of a cystamine modified poly(PFP) film (a) before (solid line, offset by 1000 counts for clarity) and (b) after (dotted line) immersion in an aqueous 10 mM  $K_2PtCl_4$  solution for 90 minutes. Solid and dashed lines of spectrum (b) correspond to the envelope and individual component fits of S (2p) doublets (the separation of components in a doublet is constrained to 1.18 eV, with relative intensity 2:1). Binding energy corrected to C (1s) at 285.0 eV, take-off angle  $90^\circ$ .

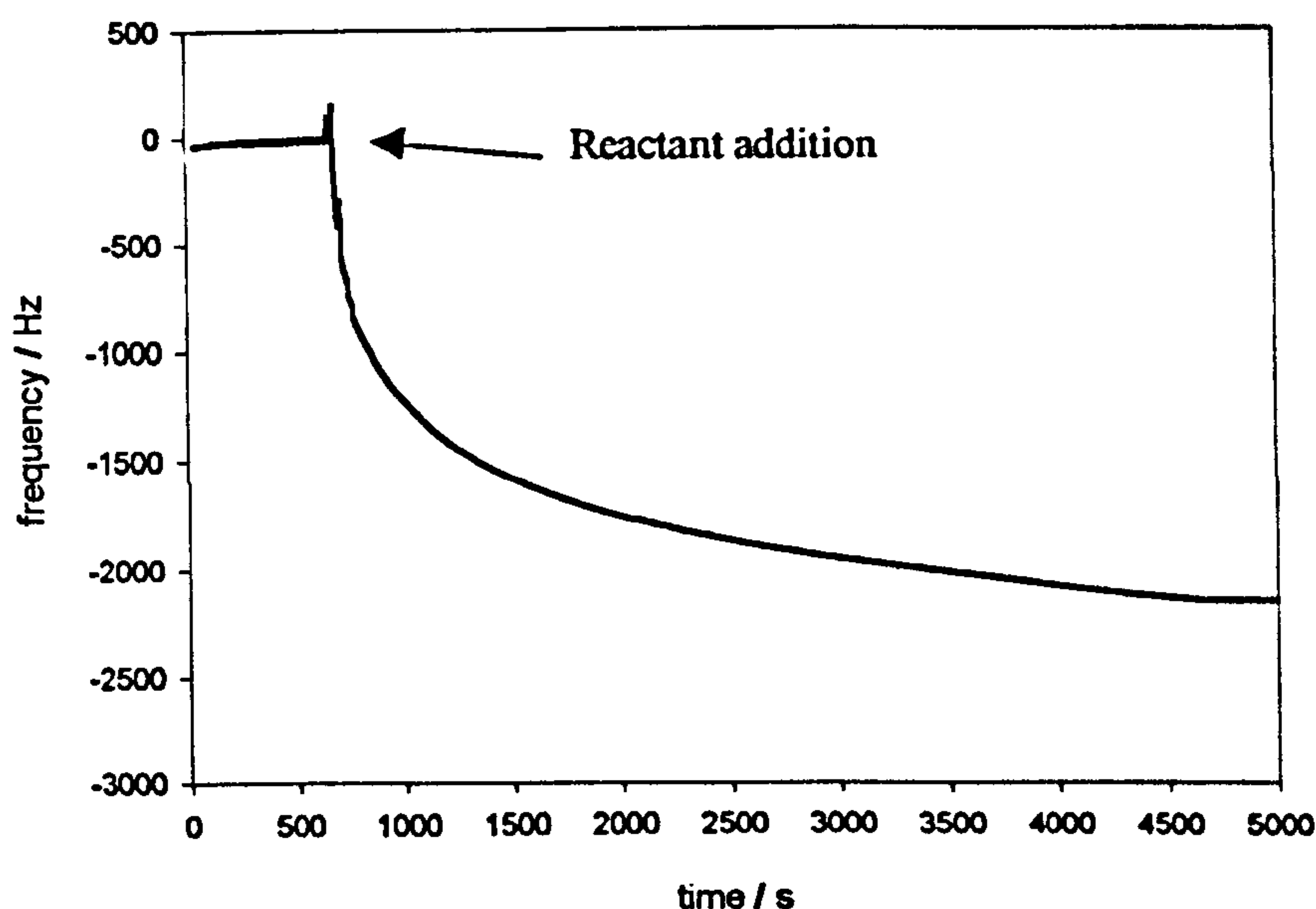
Complete quantification of the XPS measurements of Cl (2p), Pt (4f), S (2p) and O (1s) regions suggested the surface Pt: Cl: S: O ratio of the complex is 1: 1: 3: 3. This



indicated that for every Pt centre there were three sulphur centres, from the cystamine functionalised polymer. As mentioned before, two of the three S centres were weakly co-ordinated to the platinum. The oxygen present within the surface ratio was from the amide group present before Pt immobilisation. The lack of increase of oxygen content, which was indicated after analysis of the O (1s) spectrum suggests that no water was incorporated within the co-ordination complex, a possibility due to the nucleophilic properties of the water molecules.

For QCM measurements to determine the kinetics of Pt immobilisation, cystamine functionalised PFP polymer coated quartz crystals were used. The polymer-coated quartz crystals were reacted with 10 mM aqueous  $K_2PtCl_4$  and the resultant frequency changes were measured. QCM measurements performed with the system showed that the increase in mass of the cystamine modified polymer film following immersion in  $K_2PtCl_4$  solution (Figure 6.12). The large frequency change occurred within minutes after immersion of Pt containing solution. Dry QCM measurements performed on the cystamine modified poly(PFP) films treated with the  $PtCl_4^{2-}$  showed that an overall decrease in mass of the polymer compared to the unreacted poly(PFP) (approximately 5-10% or 0.45 kHz for this film). This decrease was consistent with the XPS derived ratio suggesting that the Pt complex is chelated by three sulphur species from the polymer backbone is:  $[Py-cys]_3PtCl$  has a molecular weight of  $818\text{gmol}^{-1}$  in comparison to  $303\text{gmol}^{-1}$  for one poly(PFP) unit (= 909 for 3 PFP units). Therefore, the ratio obtained from the XPS measurement was an indication of the ratio throughout the bulk composition.





**Figure 6.12** QCM frequency changes (mass increase) for cystamine modified poly(PFP) film on immersion in 10 mM  $\text{K}_2\text{PtCl}_4$  in water. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $0.229 \text{ cm}^2$ . This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.

#### 6.2.2.2 Subsequent Immobilisation Forming Platinum Complex

To initially investigate the strength of chelation of the platinum complex to the cystamine modified poly(PFP) polymer a series of potential reactions with solution based thiol and amine ligands were carried out to determine whether there is any tendency for the removal ('plucking') of platinum complex from the thiol sites within the polymer. Samples were soaked in acid, alkali and de-ionised water to establish if they have the ability to remove the platinum complex from the polymer. This latter point is an important feature to investigate, as it is desirable for a template for biomolecule immobilisation to be physically and chemically stable over a wide pH range independent, therefore, allowing the use of arbitrary solution pH's for the immobilisation of biological species. Ligands chosen to probe either the secondary immobilisation or "plucking" reactions were ethanolamine, iodobenzylamine,

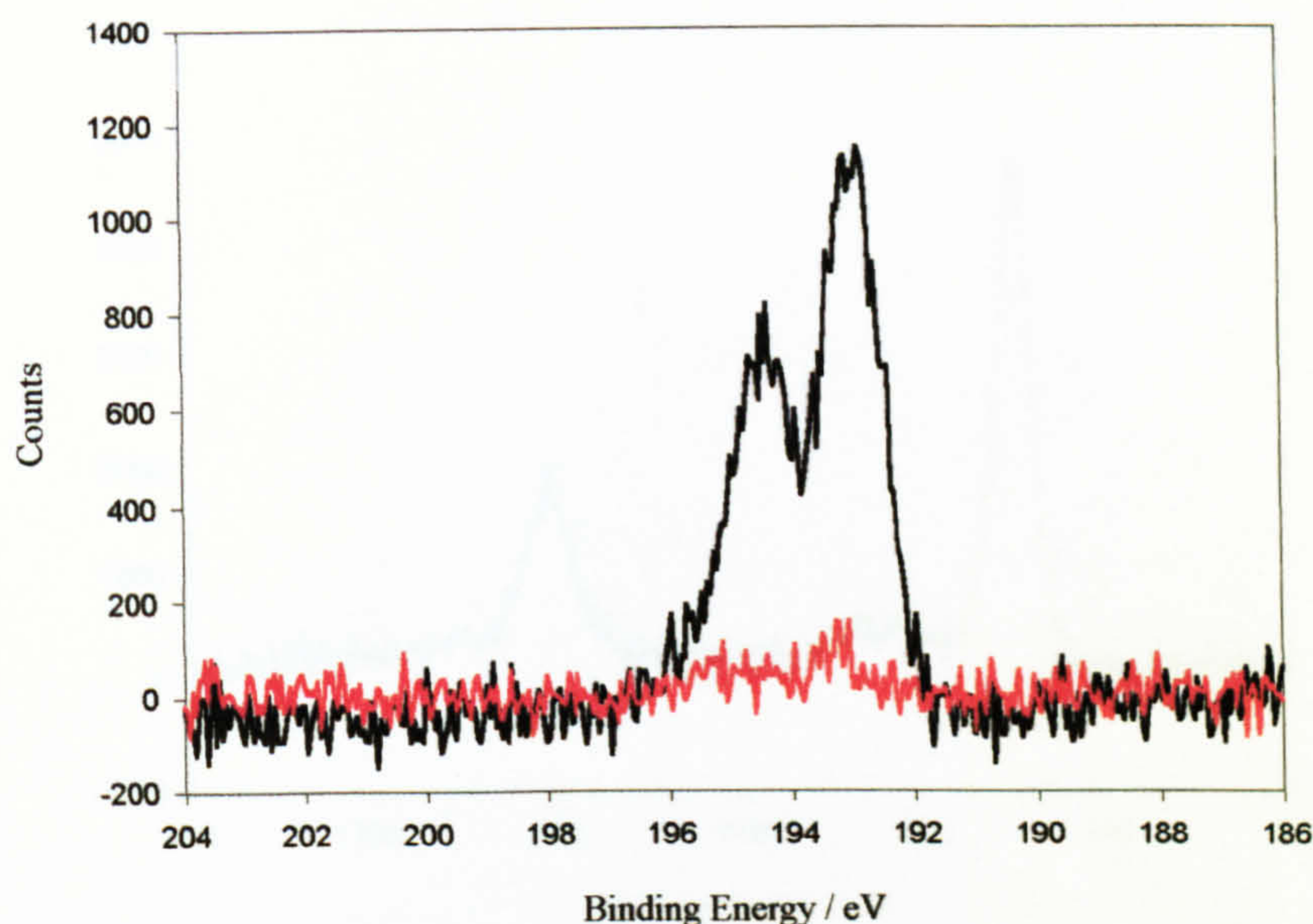


fluorothiophenol, ferrocene ethylamine and cystamine. These ligands were readily detected using XPS through the presence of either halogen, metal, nitrogen or sulphur centres. In addition, another indication of chelation to the Pt site would be a decrease in chlorine species from the platinum centres on the modified membrane.

To establish whether the surface composition of the Pt-cystamine modified electrodes was susceptible to reaction with acidic (pH 2.0), neutral (pH 7.4) and alkaline (pH 10) solutions, the samples were soaked in a specified solution. XPS analysis of the Pt (4f) and Cl (2p) regions, show that the amount of Pt and Cl present stays constant before and after immersion in the various solutions. This establishes that the system was not affected by pH conditions and, therefore, presented a potential template for the immobilisation of various biological species, which need to be reacted at a particular pH.

It was also important to determine if ligating solutions have the ability to remove platinum from its complex with polymeric cystamine. The first reaction performed was with the modified electrode and ethanolamine solution. Ethanolamine does not have a significant label to allow simple detection of its presence but XPS analysis of the sample after being immersed in the ethanolamine solution for significant period of time, showed that there is no decrease in platinum content on the sample interface. This indicated that the platinum-thiol complex was suitably robust to withstand the affect of other ligating material. In addition there was a significant decrease in chlorine content suggesting that substitution reaction has occurred to allow the attachment of the amine to the platinum centre (Figure 6.13).

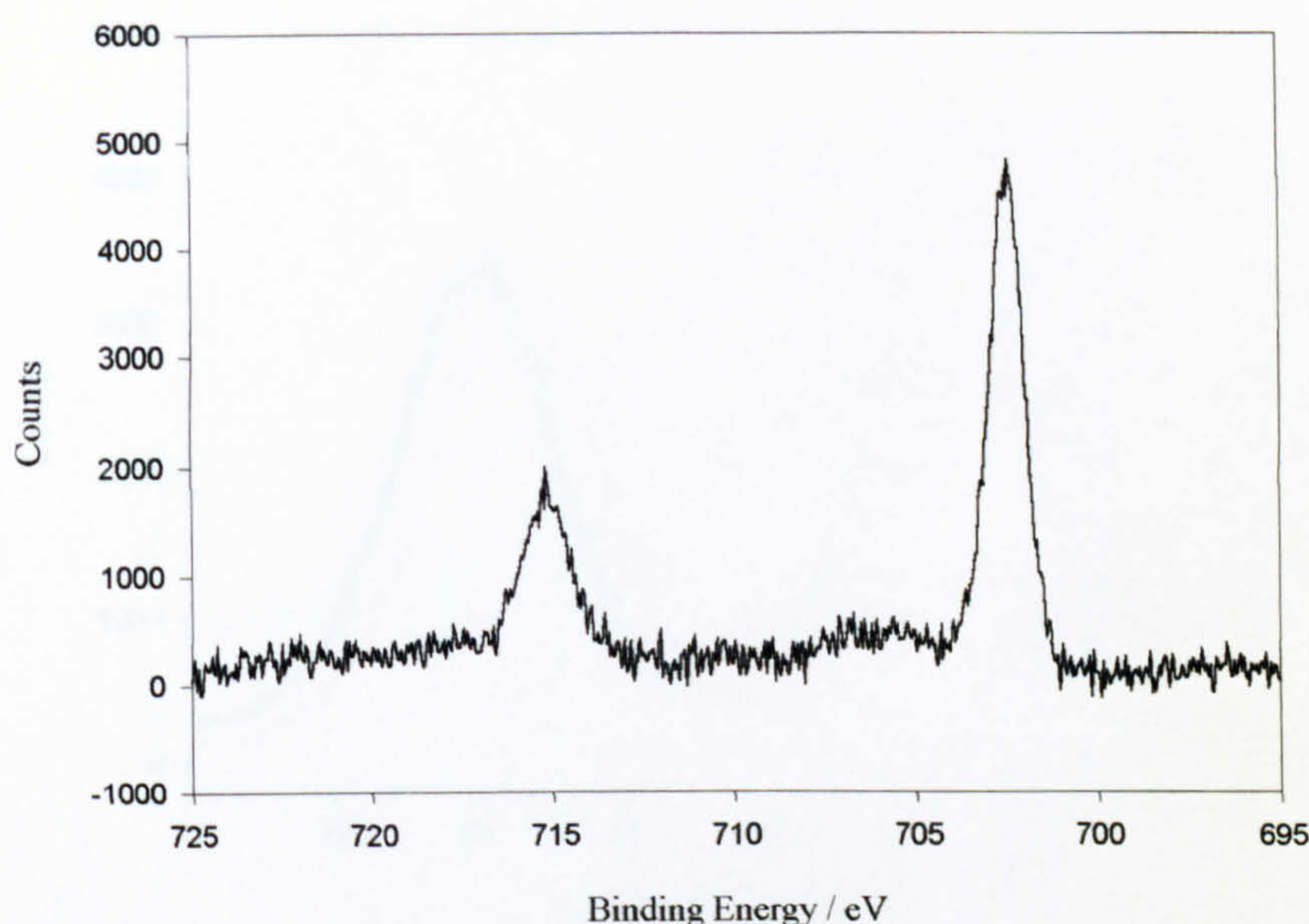




**Figure 6.13:** Cl XPS spectra (TOA 90 degrees) of cystamine-platinum modified polymer film before (black) and after (red) reaction with 1 mM ethanolamine solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $0.229 \text{ cm}^2$ . This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.

As a suitable Fe (2p) spectroscopic label was present in ferrocene ethylamine, this was the next amine used in the studies of the nucleophilic reaction with this system. The success of this reaction was indicated by the presence of the Fe (2p) doublet, shown in Figure 6.14, which suggested the success of the reaction. Again there was a significant decrease in chlorine content on the polymer surface, therefore, suggesting that during the nucleophilic reaction with ferrocene ethylamine, it complexes with the platinum centre and the chlorine acts as a leaving group for the reaction.



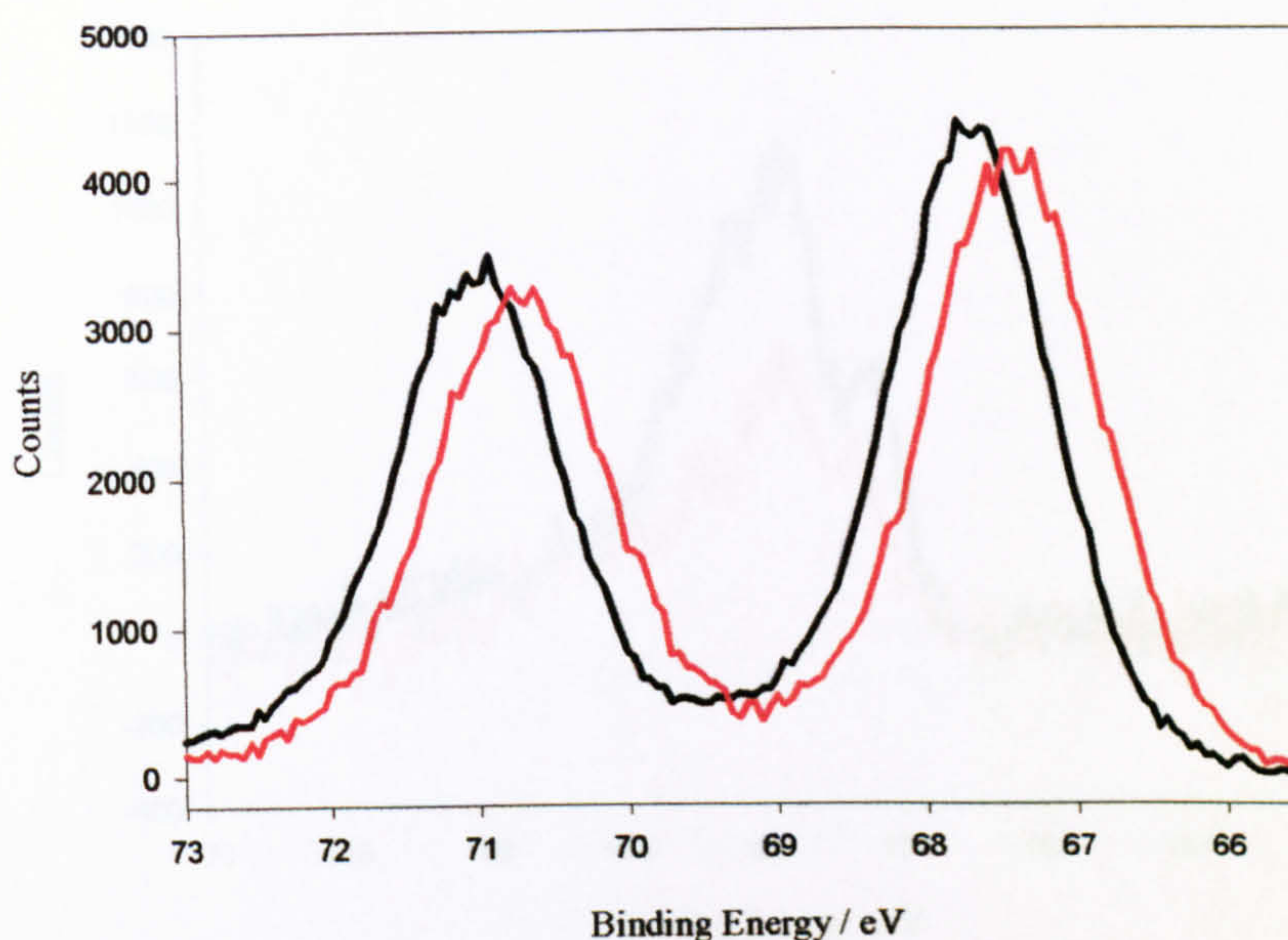


**Figure 6.14:** Fe (2p) XPS spectra (TOA 90 degrees) of cystamine-platinum modified polymer film after reaction with 1mM ferrocene ethylamine/ DMSO solution. The cystamine-platinum modified film was produced by reacting the poly(PFP) with 1 mM cystamine and DMSO solution followed by reaction with aqueous 10 mM  $K_2PtCl_4$  solution.

Moreover, on analysis of the Pt (4f) spectrum, there was a small shift of approximately 0.5 eV in the position of the doublet, giving evidence that there has been a change in the ligands attached to the platinum centre, Figure 6.15.

In addition, cystamine was also reacted with the cystamine-platinum complex. The detection of cystamine was possible due to the S (2p) signal in XPS, therefore, if cystamine successfully co-ordinates to the platinum then this would result in an increase in signal. This increase was apparent in Figure 6.16. As seen previously there was also the typical decrease in chlorine content and also a shift in position of the platinum peak, confirming that a reaction with the polymer has taken place.



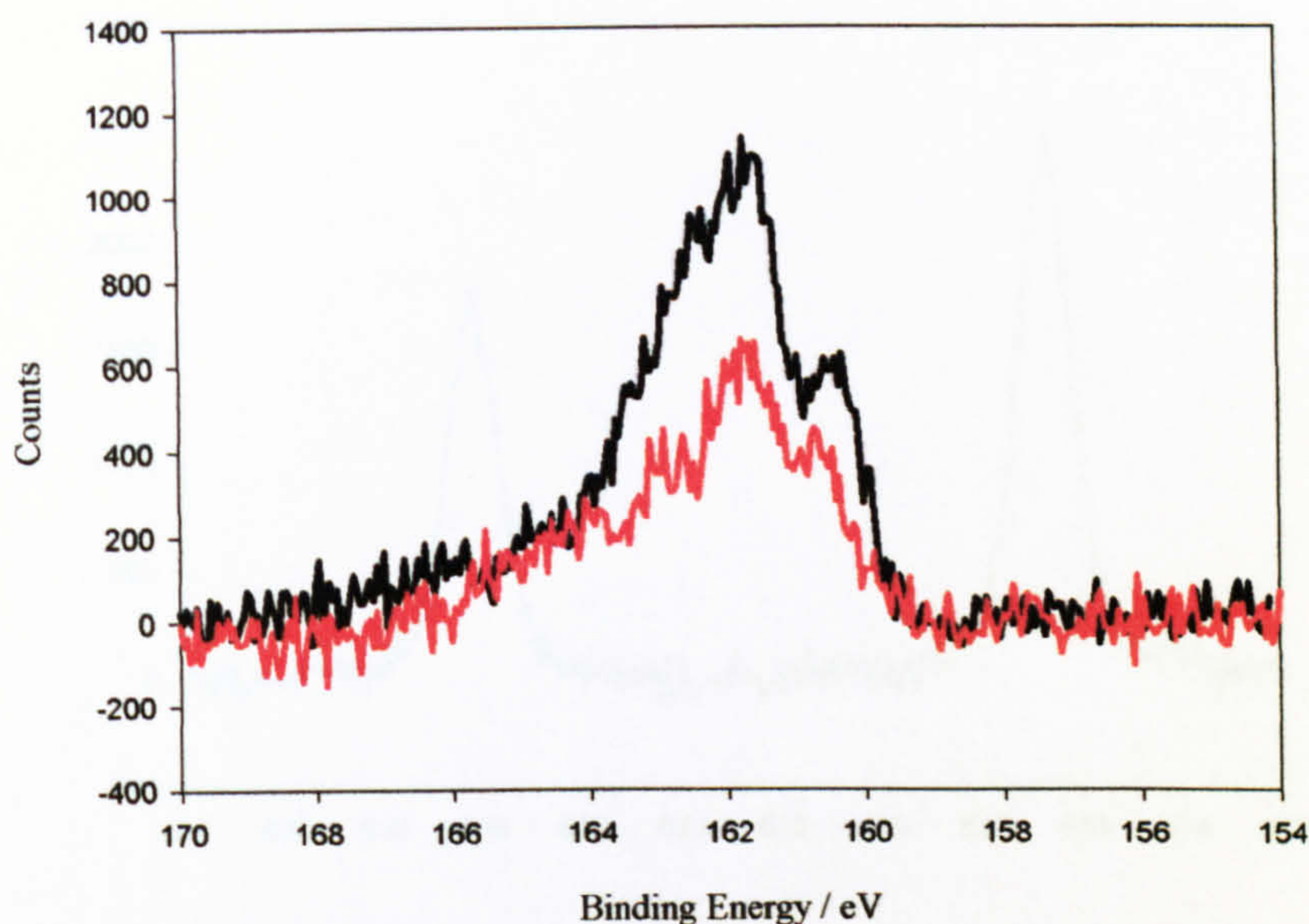


**Figure 6.15:** Pt (4f) XPS spectra (TOA 90 degrees) of cystamine-platinum modified poly(PFP) polymer before (black) and after (red) reaction with nucleophilic solution. The cystamine-platinum modified film was produced by reacting the poly(PFP) with 1 mM cystamine and DMSO solution followed by reaction with aqueous 10 mM  $K_2PtCl_4$  solution.

Finally, the reaction the platinum containing polymer layer was with the halogen-labelled species, Iodobenzylamine and fluorothiophenol. The I (3d) label and F (1s) can act as a distinct probe for the reaction. Again, there is a decrease in chlorine content and also a shift in the position of the Pt doublet, compared to the unreacted sample. In Figure 6.17, the presence of Iodine is indicated by the appearance of the I (3d) doublet after immersion in the iodobenzylamine solution. A F(1s) signal was also observed after reaction with fluorothiophenol (Figure 6.18).

Ultimately, the complex formation with the square planar platinum centre has proved successful and could, therefore, in the future be used as a template for immobilisation of biological species, which are amine terminated or possibly with a thiol reactive group.

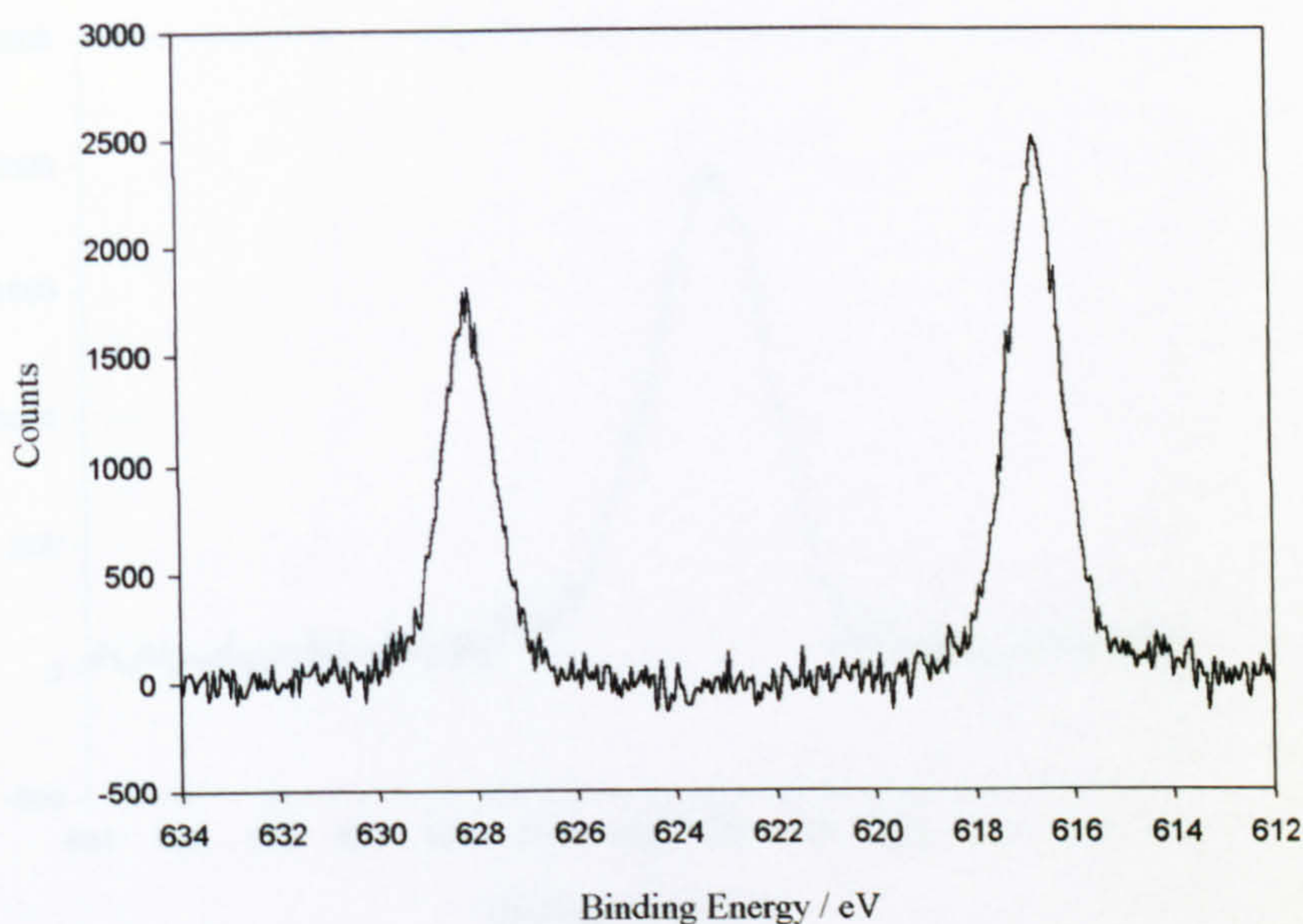




**Figure 6.16:** S (2p) XPS spectra (TOA 90 degrees) of cystamine-platinum modified poly(PFP) film before (red) and after (black) reaction with 1 mM cystamine/ MeCN solution. The cystamine-platinum modified film was produced by reacting the poly(PFP) with 1 mM cystamine and DMSO solution followed by reaction with aqueous 10 mM  $K_2PtCl_4$  solution.

Quantification of the XPS measurements for Pt (4f), S (2p) and the labels from the newly attached ligands; Fe (2p), I (3d) and F (1s), show surface ratios of 1: 3: 1 for each sample. In addition, analysis of the XPS spectra shows that there was no chlorine present in each sample. The derived ratio suggests that for each of the chelated platinum centres there is one ligand attached by substitution of the remaining chlorine atom.



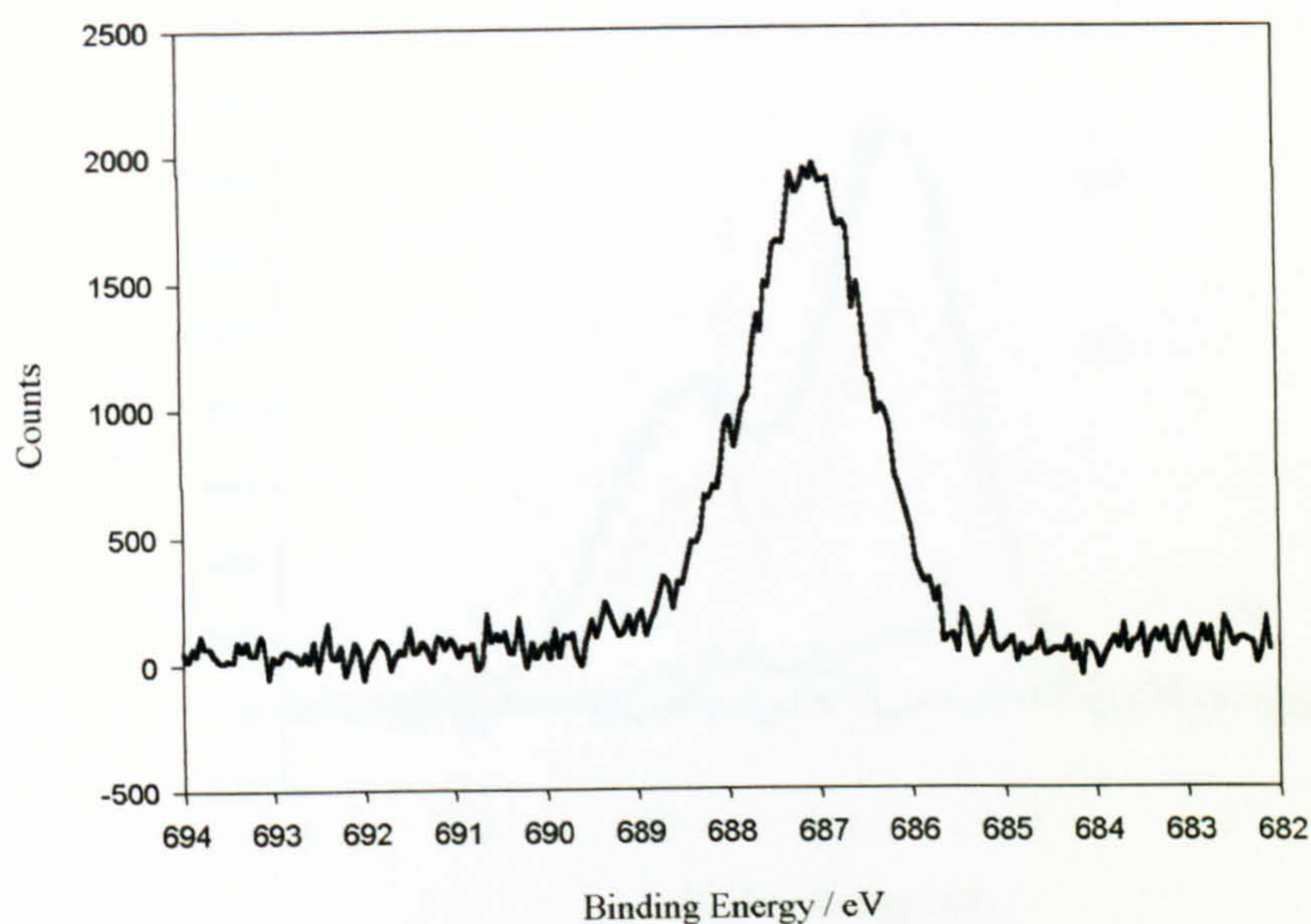


**Figure 6.17:** I (3d) XPS spectra (TOA 90 degrees) of cystamine-platinum modified poly(PFP) film after reaction with 1 mM Iodobenzylamine and buffer solution. The cystamine-platinum modified film was produced by reacting the poly(PFP) with 1 mM cystamine and DMSO solution followed by reaction with aqueous 10 mM  $K_2PtCl_4$  solution.

### 6.2.3 Potential Control of Poly(PFP) Reaction with Cystamine

To determine whether electrode potential control can be used to successfully inhibit cystamine immobilisation, electropolymerised films of poly(PFP) were immersed in acetonitrile based solutions containing supporting electrolyte (TEAP) and oxidatively doped prior to immersion in a cystamine solution. The XPS results shown in Figure 6.19 clearly shows that the incorporation of cystamine species at the polymer-solution interface was inhibited by maintaining the polymer in a doped state. In Figure 6.18, comparison of the S (2p) XPS spectra (a) and (b) showed that the amount of cystamine in the surface layers of the film following immersion of a doped electrode in the cystamine solution was negligible comparable to that immobilisation on an undoped polymer. Importantly, this inhibition is removed following the undoping of the same polymer.

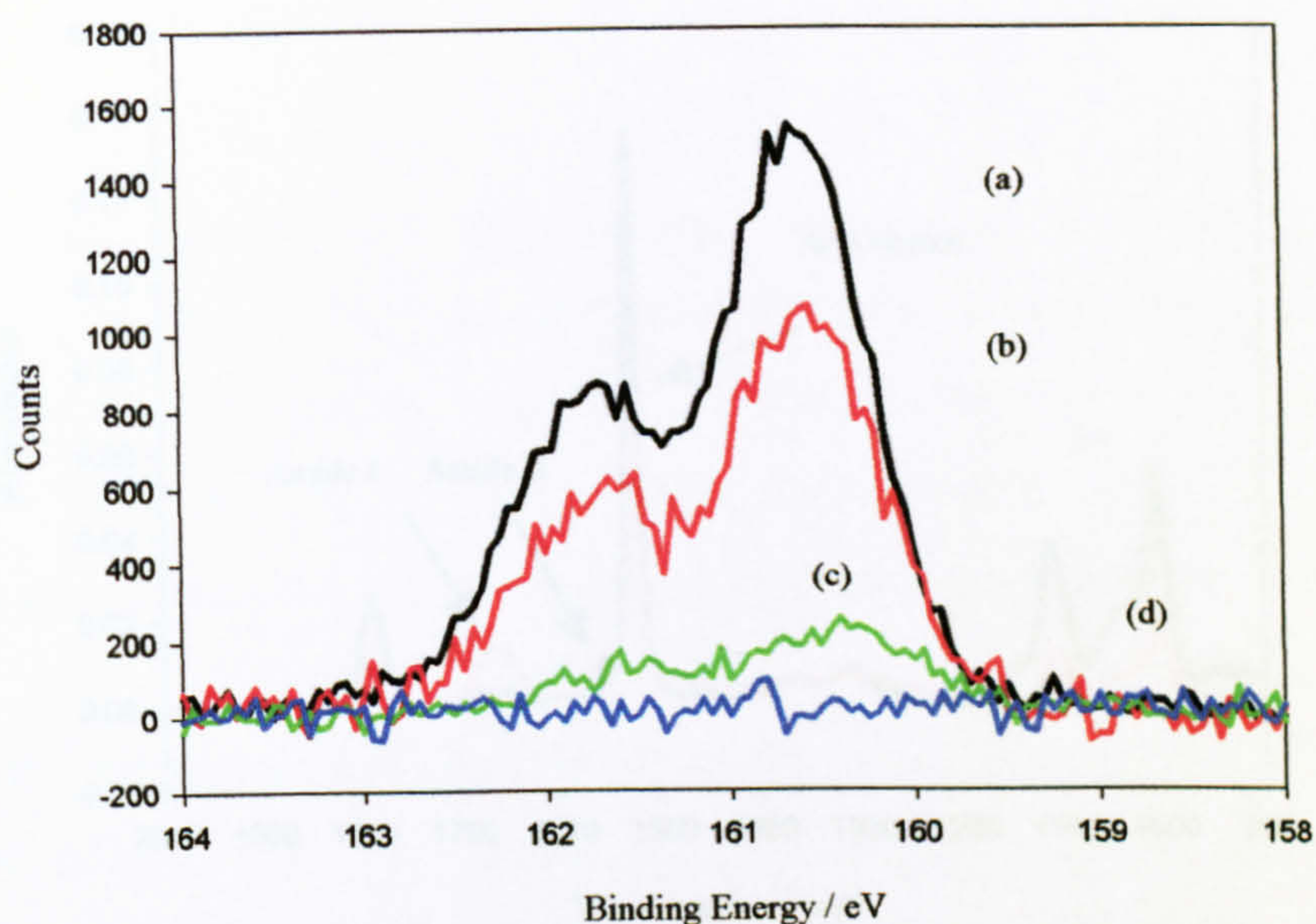




**Figure 6.18:** F (1s) XPS spectra (TOA 90 degrees) of cystamine-platinum modified poly(PFP) film after reaction with fluorthiophenol solution. The cystamine-platinum modified film was produced by reacting the poly(PFP) with 1 mM cystamine and DMSO solution followed by reaction with aqueous 10 mM  $\text{K}_2\text{PtCl}_4$  solution.

It was observed that the extent of nucleophilic reaction was also reduced when an undoped poly(PFP) film was immersed in cystamine solutions containing 0.1 M or 0.2 M TEAP electrolyte (Figure 6.19 (b) and (c)). Since the oxidation of the polymer also involved the increase in  $\text{ClO}_4^-$  (electrolyte) concentration within the film, it was postulated that this high salt solution concentration environment may partially mimic the oxidised state of the doped polymer film as a consequence of salt partitioning into the polymer.



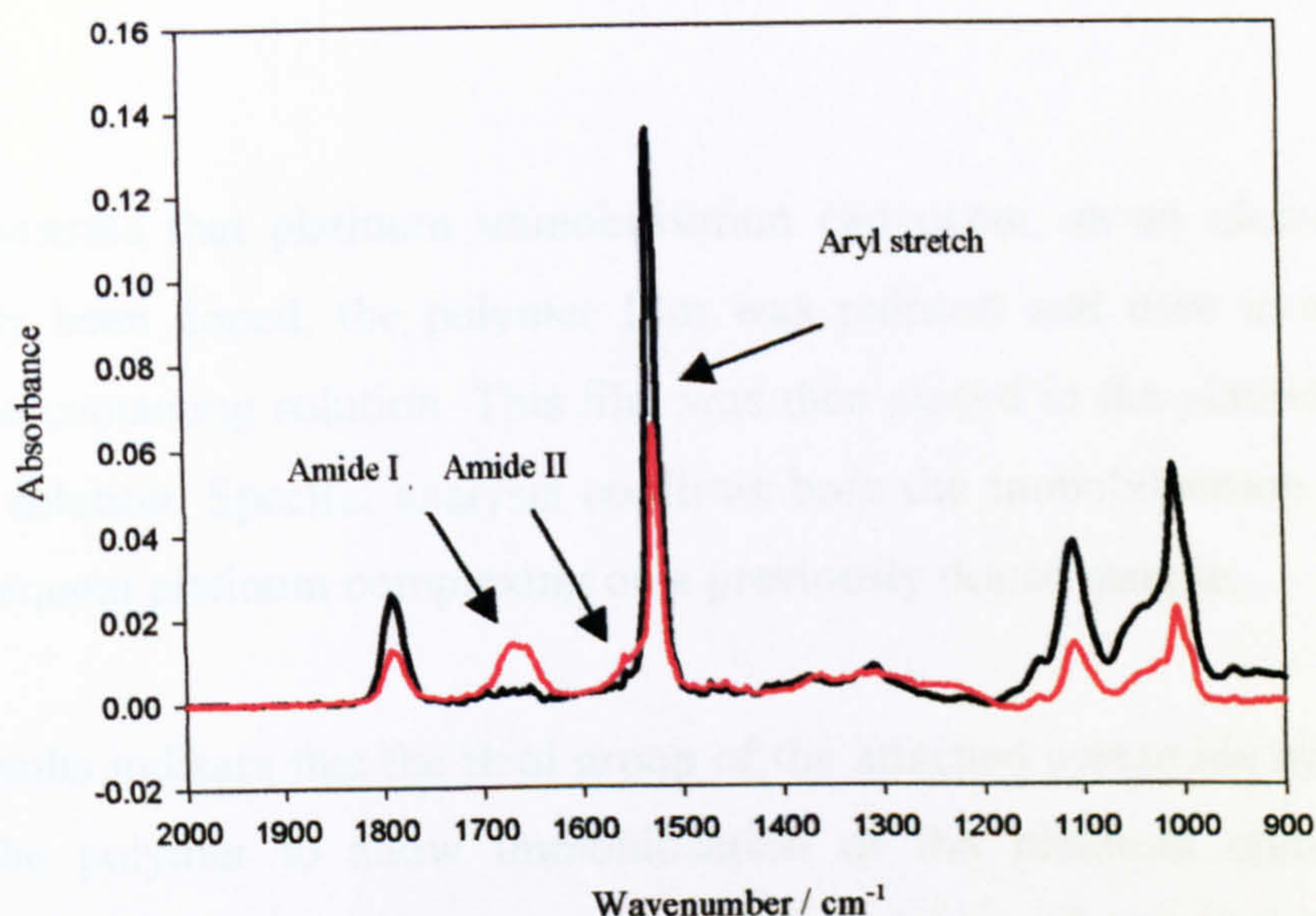


**Figure 6.19:** S (2p) XPS spectra (TOA 90 degrees) of undoped (black) and doped (blue) poly(PFP) films after immersion in 1 mM cystamine, DMSO solution for 5 minutes. The S (2p) XPS spectra of poly(PFP) after immersion in 1 mM cystamine and DMSO solution for 5 minutes, containing 0.1 M TEAP (red) and 0.2 M TEAP (green) respectively are shown. The poly(PFP) films were from solution containing 20 mM PFP, 0.1 M TEAP and MeCN using a scan rate of  $20 \text{ mVs}^{-1}$  with electrode area of  $1 \text{ cm}^{-2}$ .

#### 6.2.4 Control of Formation of Cystamine and Polymer Complex

Taken by themselves, the results in Figure 6.19 do not distinguish between cystamine species have become covalently attached to the polymer as a result of nucleophilic substitution or simply adsorbed onto the surface. However, Figure 6.20 shows the RAIRS absorbance spectra for a deposited poly(PFP) film and a doped poly(PFP) film after immersion in cystamine. The spectra of the undoped film after immersion shows a partial loss of the fluorophenol ester peak at  $1790 \text{ cm}^{-1}$  and with the appearance of amide I and amide II peaks, at  $1680 \text{ cm}^{-1}$  and  $1540 \text{ cm}^{-1}$  (although difficult to see due to the remaining aryl stretch). Significantly, there was little change between the unreacted PFP spectrum and the spectrum of the doped polymer after solution immersion. Thus, it was concluded that the cystamine present in the film after the appropriate electrochemical reaction conditions has been immobilised via amide bond formation as a result of nucleophilic substitution.





**Figure 6.20:** RAIRS absorbance spectra for unreacted poly(PFP) films after immersion in 1 mM cystamine and DMSO solution at specified electrochemical conditions: (a) oxidatively doped poly(PFP) (black) and (b) undoped poly(PFP) (red).

#### 6.2.4 Control of Formation of Cystamine and Platinum Complex

Due to the success of being able to control the reactivity of the activated ester in poly(PFP) with cystamine, further experiments were carried out to attempt to selectively immobilise the platinum complex ( $\text{PtCl}_4^-$ ). An aim of these experiments is to determine whether this system could possibly be used as a template for the micropatterning of biomolecules after reaction with the available transition metal sites. As described in the previous section, the reaction of poly(PFP) with cystamine was inhibited by oxidatively doping the polymer backbone. Figure 6.21 shows the Pt (4f) and Cl (2p) XPS regions of a PFP electrode that had been previously immersed in a cystamine solution whilst being held in the oxidised state. Pt (4f) region indicate that there is no platinum present on the surface layers of the modified polymer membrane. The Cl (1s) spectrum also reveals that there is a lack of chlorine present



within the film, therefore, suggesting that there was no complex formed. Clearly, by comparison with the spectra of cystamine-modified electrode after reaction in the platinum containing solution (Figure 6.21), where Pt (4f) and Cl (1s) peaks are visible.

To demonstrate that platinum immobilisation can occur on an electrode that has previously been doped, the polymer film was reduced and then immersed in the cystamine containing solution. This film was then placed in the platinum containing aqueous solution. Spectral analysis confirms both the immobilisation of cystamine and subsequent platinum complexing on a previously doped sample.

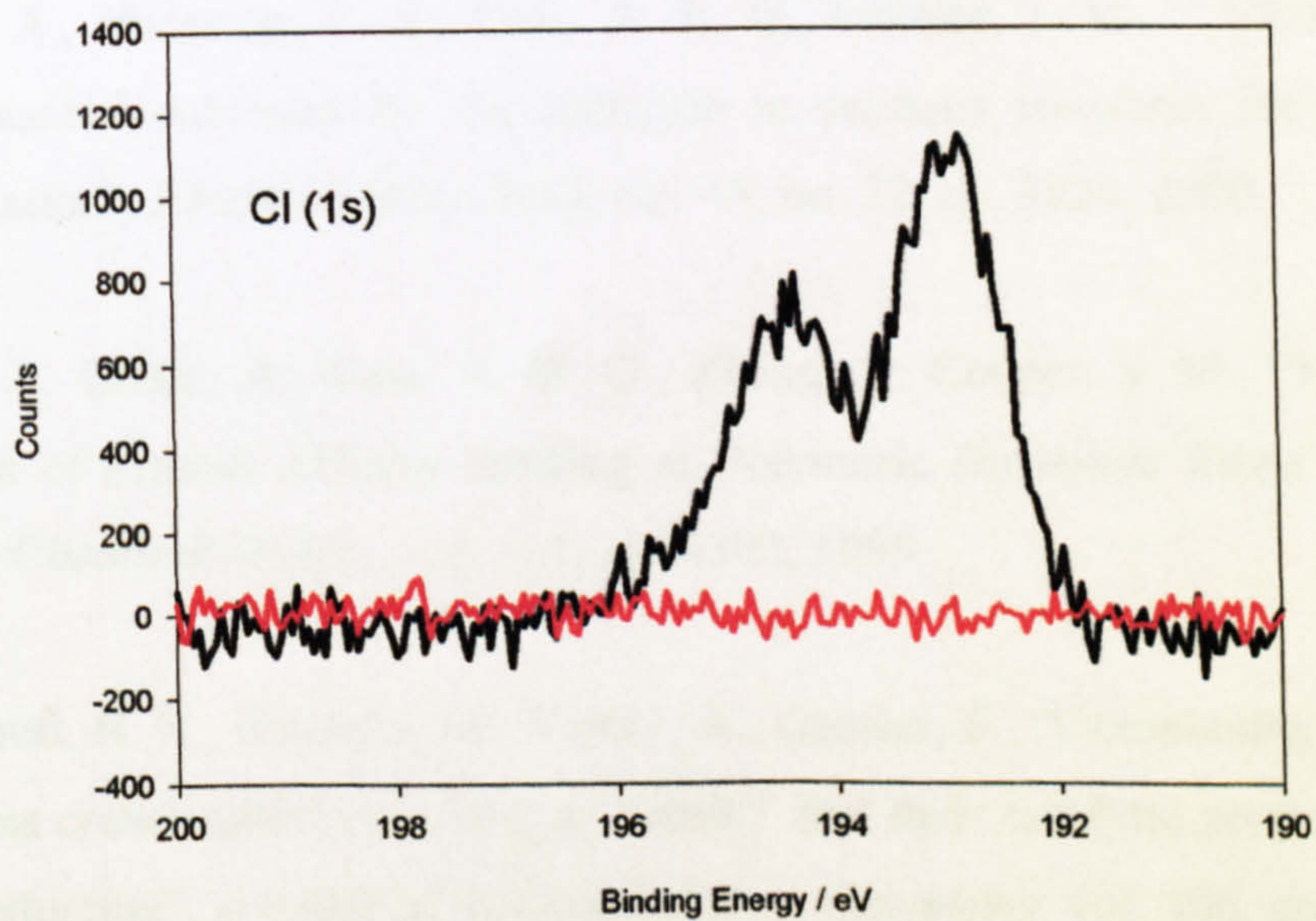
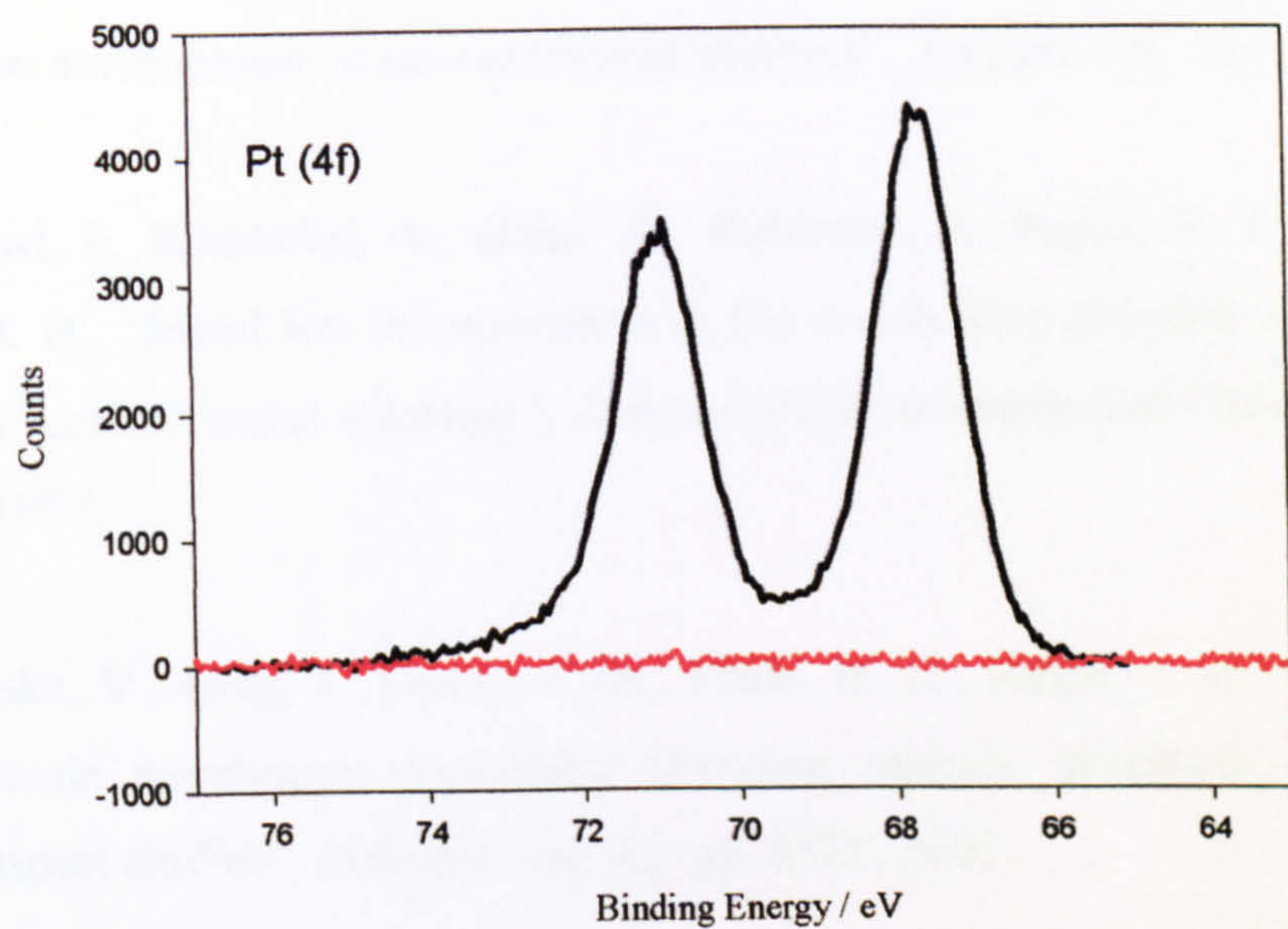
These results indicate that the thiol group of the attached cystamine must be present within the polymer to allow immobilisation of the platinum complex. On the unreacted homopolymer there was no significant signs of non-specific adsorption occurring.

### ***6.3 Summary***

XPS measurements have been used to determine an appropriate solvent, which allowed an organic coupling reaction between poly(PFP) and solution species (cystamine) to proceed. Subsequently, the cystamine-modified polymer was used as a template for metal ion chelation. A combination of XPS and QCM measurements has enabled to show that the binding of a “soft” metal species, Pt, from aqueous solution to thiol groups within the polymer is possible. The resulting complex retains chloride ligands, which allow further nucleophilic substitution with a variety of species. This was investigated using species, which have an easily detectable XPS probe, and results show substitution of the chloride with amine and thiol terminated species is possible.

Control of cystamine reaction is possible using the doping process described, which makes it possible to produce a patterned surface that could be used to immobilise specific species.





**Figure 6.21:** Pt (4f) and Cl (1s) XPS spectra (TOA 90 degrees) after reaction of 10 mM  $\text{K}_2\text{PtCl}_4/\text{H}_2\text{O}$  with doped (red) and undoped (black) poly(PFP) films that have been reacted with 1 mM cystamine/DMSO solution.



## 6.4 References

1. Lewis, T. W., Wallace, G. G., Smyth, M. R., "Electrofunctional polymers: their role in the development of new analytical systems", *Analyst*, vol. 124, pp. 213, 1999.
2. Marawi, I., Khaskelis, A., Galal, A., Robinson, J., Popat, R. P., Boerio, F. J., Mark, H. B., "Metal ion incorporation in the conducting polymer electrode matrix using an "active" metal substrate", *Journal of Electroanalytical Chemistry*, vol. 434, pp. 61, 1997.
3. Misoska, V., Ding, J., Davey, J. M., Price, W. E., Ralph, S. F., Wallace, G. G., "Polypyrrole membranes containing chelating ligands: synthesis, characterisation and transport studies", *Polymer*, vol. 42, pp. 8571, 2001.
4. Glidle, A., Hadyoon, C. S., Cass, A. E. G., Cooper, J. M., " Modifications of electrodeposited polymers by ion chelation to produce templates for biomolecule immobilisation", *Electrochimica Acta*, vol. 45, no. 22, pp. 3823, 2000.
5. Davis, J., Glidle, A., Cass, A. E. G., Zhang, J., Cooper, J. M., "Spectroscopic Evaluation of Protein Affinity Binding at Polymeric Biosensor Films", *Journal of American Chemical Society*, vol. 121, pp. 4302, 1999.
6. Youssoufi, H. K., Hmyene, M., Yassar, A., Garnier, F., "Complexing properties of poly[3-(aza crown ether) pyrroles] to cobalt<sup>2+</sup> and their catalytic properties towards oxygen reduction", *Journal of Electroanalytical Chemistry*, vol. 406, pp. 187, 1996.
7. Gasper, S., Habermuller, K., Csoregi, E., Schumann, W., "Hydrogen peroxide sensitive biosensor based on plant peroxidases entrapped in Os-modified polypyrrole films", *Sensors and Actuators*, vol. 72, pp. 63, 2001.
8. Henderson, R. A., The mechanism of reactions at transition metal sites, Oxford University Press, Oxford, 1993.



9. Sykes, P., A guidebook to mechanism in organic chemistry, 6<sup>th</sup> edition, Longman, London, 1986.



# Chapter 7

## *Interaction of Biotin Modified Poly(PFP) Films with Streptavidin and Streptavidin-Enzyme Conjugates*

### *7.0 Introduction*

Due to the fact that avidin and streptavidin bind to biotin selectively with an extremely high affinity, this interaction has previously been used in a wide range of systems e.g. pre-targeting of malignant tumours, immunohistochemistry, enzyme-linked immunoassays and molecular biology<sup>1-4</sup>. The high binding strength and specificity is a consequence of aromatic and hydrogen bonding between biotin and a pocket in the tertiary structure of streptavidin (or avidin). The shape of this pocket is such that it specifically accommodates biotin motifs. This high affinity and selectivity has prompted research to develop this system for biosensor technology<sup>5-9</sup>. The research detailed within this chapter concerns the development of immobilisation schemes for the attachment of biological motifs to polymeric matrix.

Two schemes of biotin attachment to poly(PFP) were developed in this chapter. Firstly an amine derivative of biotin was directly reacted with the activated ester present in poly(PFP). Secondly, the poly(PFP) film was reacted first with cystamine and then with I-PEO-acetylbiotin. This latter method offers the possibility of patterning using a similar strategy outlined in the previous chapter to develop a Pt template.



In this investigation, to facilitate the XPS identification of biological species such as streptavidin and conjugated enzymes, brominated or iodinated derivatives were prepared, as described in Section 7.2.

## **7.1 Materials**

**Solvents:** DMSO, MeCN and dichloromethane were obtained from Aldrich.

**PFP and TEAP** were prepared as described in Chapter 2.

**5-(Biotinamido) pentylamine** (Pentylamine Biotin) was obtained from Amphotech Ltd., Beverly, MA 01915, USA.

**EZ-Link PEO-Iodoacetyl Biotin** {(+)-Biotinyl-iodoacetamidyl-3, 6-dioxaoctanediamine} (I-PEO-acetylbiotin) was obtained from Pierce, Rockford, IL 61105, USA.

**Biomolecules:** Streptavidin (St), streptavidin-alkaline phosphatase (St-AP) and streptavidin- hydrogen peroxidase (St-HRP) were obtained from Sigma.

### **Labelled streptavidin and streptavidin conjugates:**

Iodination using 1,3,4,6-tetrachloro-3 $\alpha$ , 6 $\alpha$  diphenyl glycouril (Iodogen obtained from Sigma) was performed using established procedures<sup>10</sup>. This involved dissolving Iodogen in dichloromethane and placing into a suitable container. The dichloromethane was allowed to evaporate and then 0.1 ml<sup>-1</sup> streptavidin and KI solution was added to the container. The mixture was left for 2 hours and then the solution was decanted from the container. This was then carefully placed in a 10,000 MW dialysis cassette (cassettes were obtained from Pierce, Rockford, IL 61105, USA) and placed in a beaker containing buffer solution to remove the excess labelling reagents.

Bromonation was also performed by adding a 1 mM aqueous solution of NaBr and 1 mM N-Bromosuccinamide (obtained from Sigma) to the biomolecule solution (1 ml

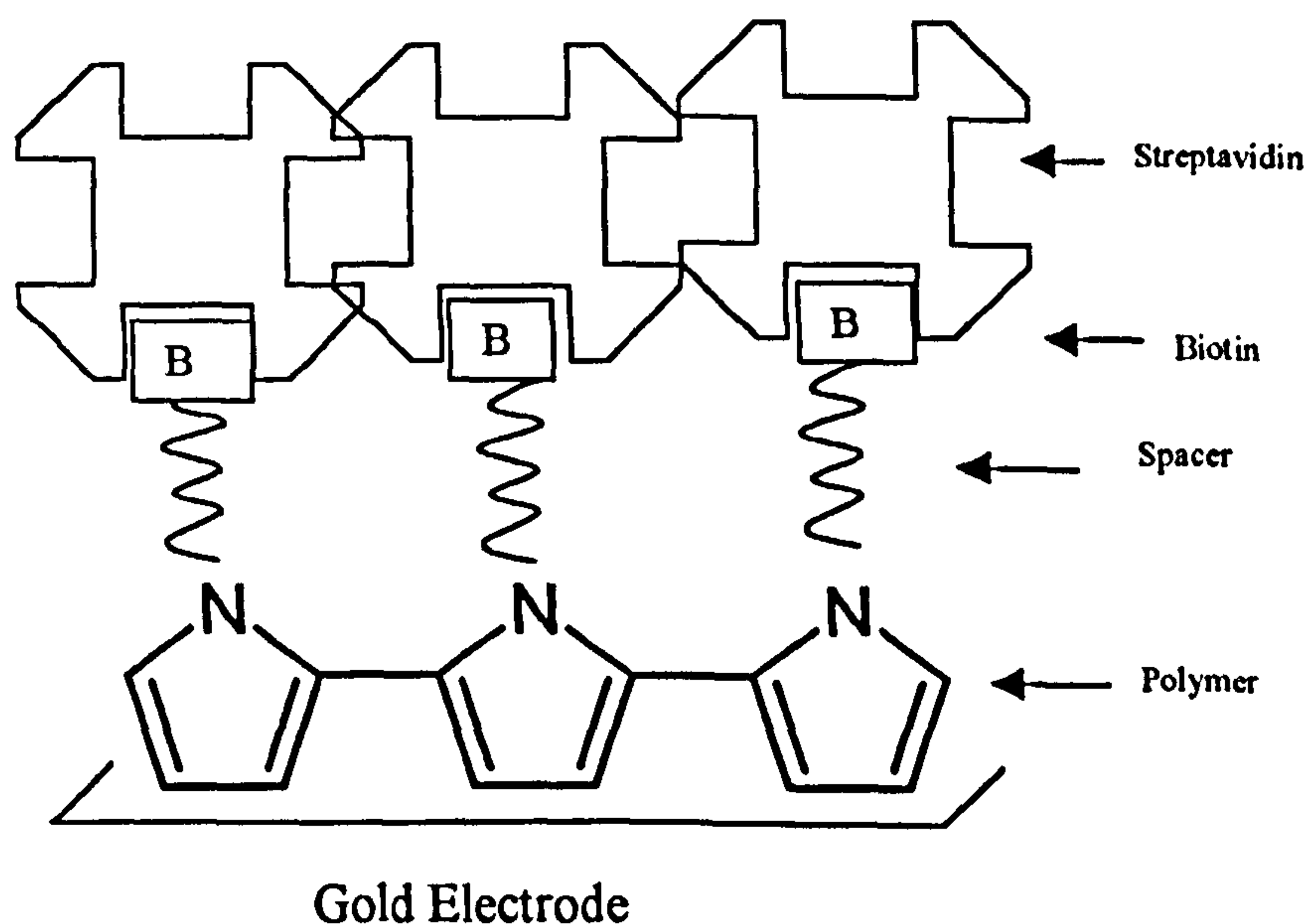


bromide solution was added to 1 ml<sup>-1</sup> biomolecule solution). A reaction time of ca. 1 hour was used with these methods and importantly, in all cases, excess labelling reagents were removed by dialysis, using 10,000 MW dialysis cassettes.

## 7.2 Results and Discussion

### 7.2.1 Reaction of Biotin Modified Poly(PFP) Film with Streptavidin

These investigations initially involved the immobilisation of a biotinylated species on the homopolymer of PFP. This reaction was confirmed using RAIRS (used to detect amide bonds and carbonyls present in biotin derivative) and XPS (detection of S (2p) signal from biotin). Subsequently, the specific interaction of the biotinylated polymer with streptavidin species was investigated, using XPS analysis, to establish whether a multicomponent system can be developed to immobilise enzymes.



**Figure 7.1:** Schematic diagram illustrating the immobilisation of biotin on pyrrole polymer and the subsequent interaction with streptavidin molecules.



### 7.2.1.1 Reaction of Homopolymer with Pentylamine Biotin

The structural formula of pentylamine biotin, in Figure 7.2, shows the presence of sulphur within the structure. Thus when performing XPS measurements, this sulphur centre can be used as a characteristic label to determine whether immobilisation was successful. The biotinylated polymer was formed by reaction of an amine terminated biotin derivative with the activated ester present within the poly(PFP) film (Figure 7.3). The experimental elemental ratio after reaction is close to C:N:S:O 22:3:1:4, which is the theoretical ratio for fully reacted poly(PFP) film. The tethering of the biotin to the polymer is also characterised using RAIRS to detect both amide bond formations within the bulk composition and characteristic peak corresponding to the imide and ester carbonyls present in the biotin motif.

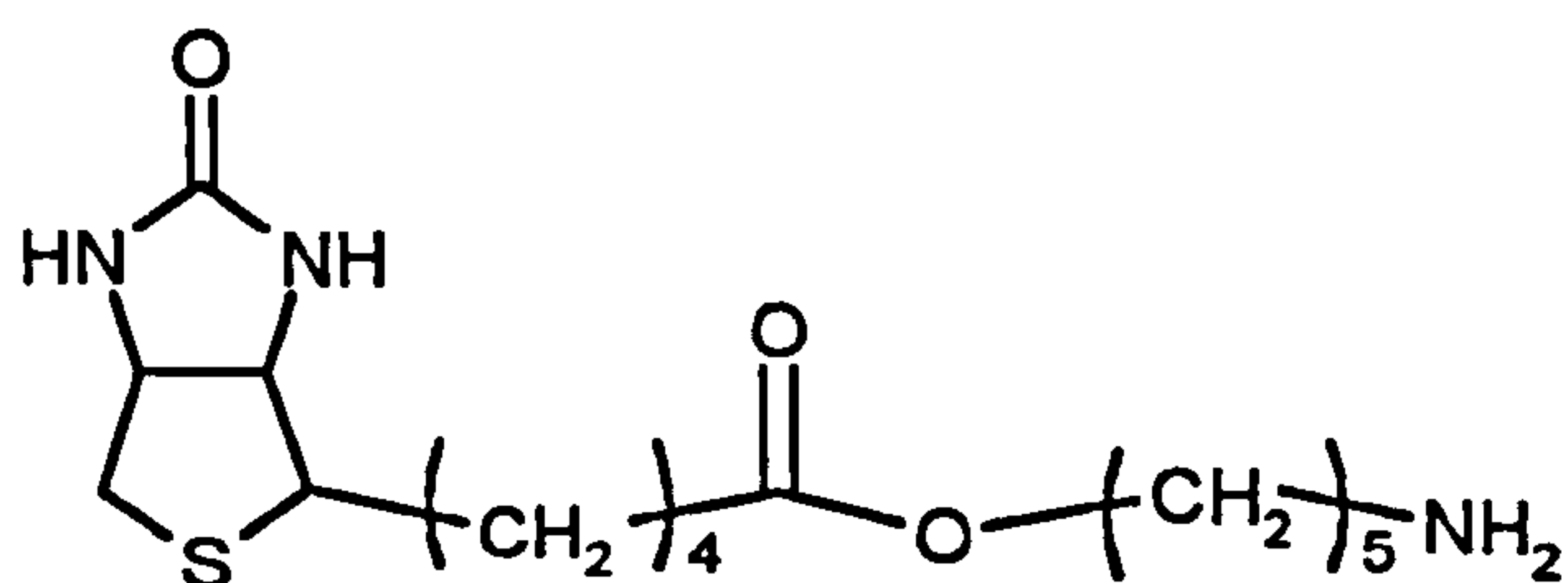


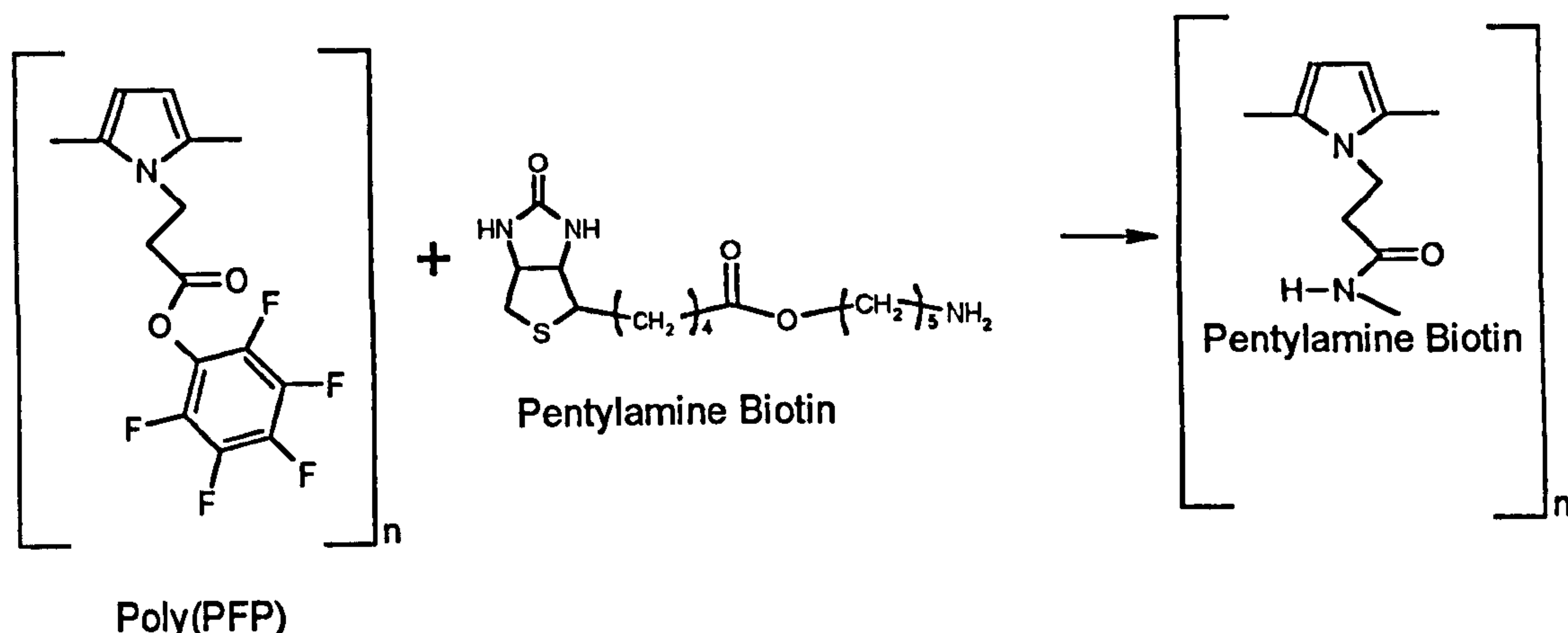
Figure 7.2: Structural formula of pentylamine biotin.

Figure 7.3 shows the reaction scheme of pentylamine biotin with poly(PFP). Again the amine reacts with the activated ester within the polymer. As a result amide bonds are formed and are detected by RAIRS. Figure 7.4, shows peaks between 1670 and 1540  $\text{cm}^{-1}$  confirming the formation of amide bonds. The peak present at 1700  $\text{cm}^{-1}$  is a result of the characteristic stretch of a HN-CO-NH group within the biotin structure.

From the XPS spectrum collected after reaction of poly(PFP) with pentylamine terminated biotin species there was an indication of biotin immobilisation due to the appearance of S (2p) doublet, as seen in Figure 7.5. An indication that the nucleophilic reaction of the amine-terminated biotin with the activated ester has



occurred is the decrease in C-F content and increase in C-H content within the film composition as seen in the C (1s) spectra of Figure 7.5.

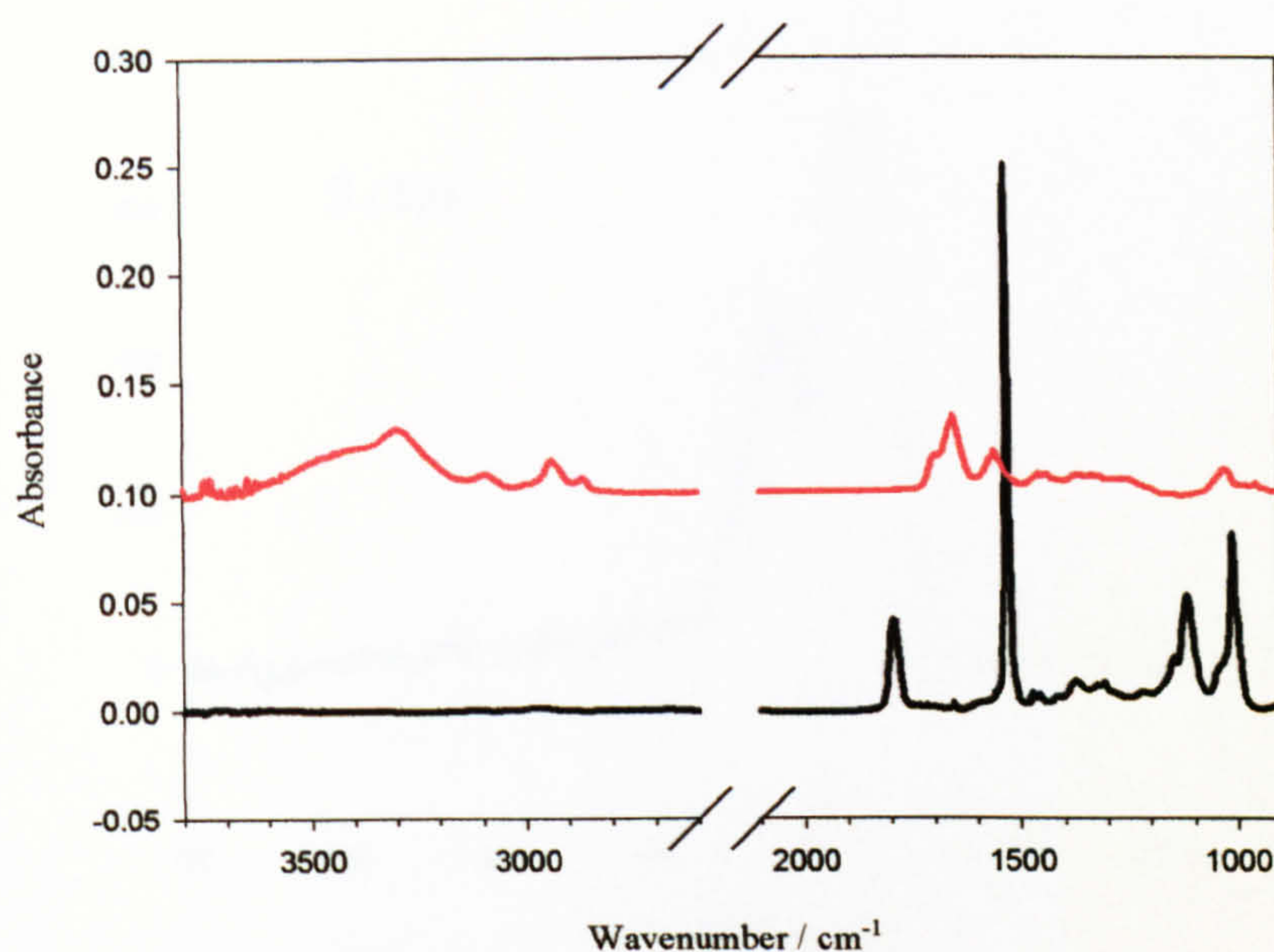


**Figure 7.3:** Reaction Scheme of poly(PFP) with pentylamine biotin.

#### 7.2.1.2 Reaction of Biotin Modified Polymer with Streptavidin

In order to evaluate the molecular recognition characteristics of the biotinylated polymer toward streptavidin species, a study using XPS was carried out. After immersion of the biotinylated polymer in an iodinated streptavidin solution, the XPS analysis shows an I (3d) doublet at 615 and 630 eV indicating the presence of iodine within the surface of the film (Figure 7.5). Control measurements of biotinylated polymer films after immersion in KI solutions showed that I<sup>-</sup> does not bind to the polymer surface. Thus the signal of Figure 7.5 shows that the iodinated streptavidin is present on the surface of the polymer.

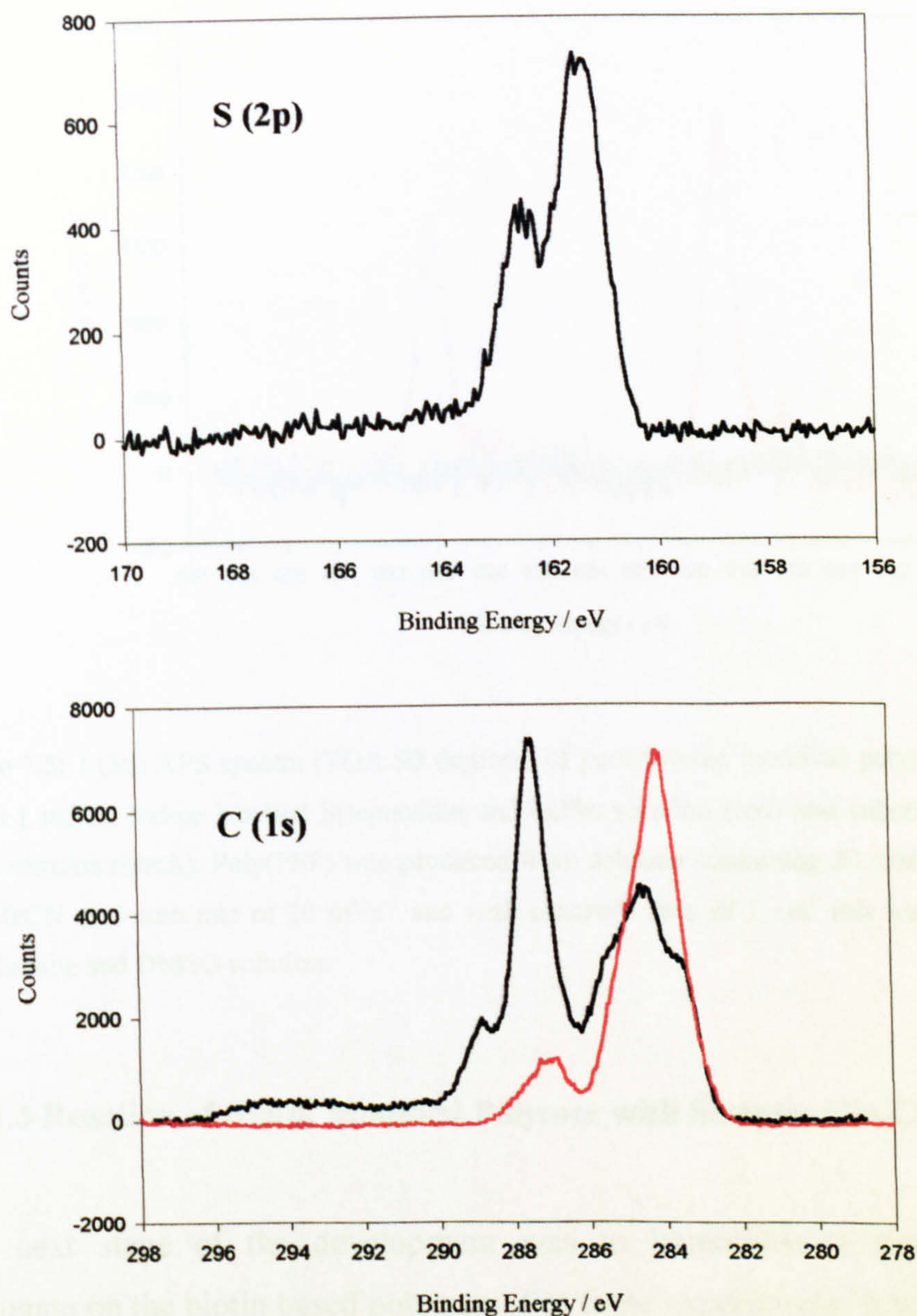




**Figure 7.4:** RAIRS spectra of unreacted poly(PFP) (black) and poly(PFP) after reaction with 1 mM pentylamine biotin and DMSO solution (red). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>. Note the presence of the amide bands at 1540 and 1690 cm<sup>-1</sup>. Also visible is a peak at 1700 cm<sup>-1</sup>, which corresponds to N-CO-N group incorporated within a 5 ring system. In addition C-H stretches are visible at 3000 cm<sup>-1</sup>.

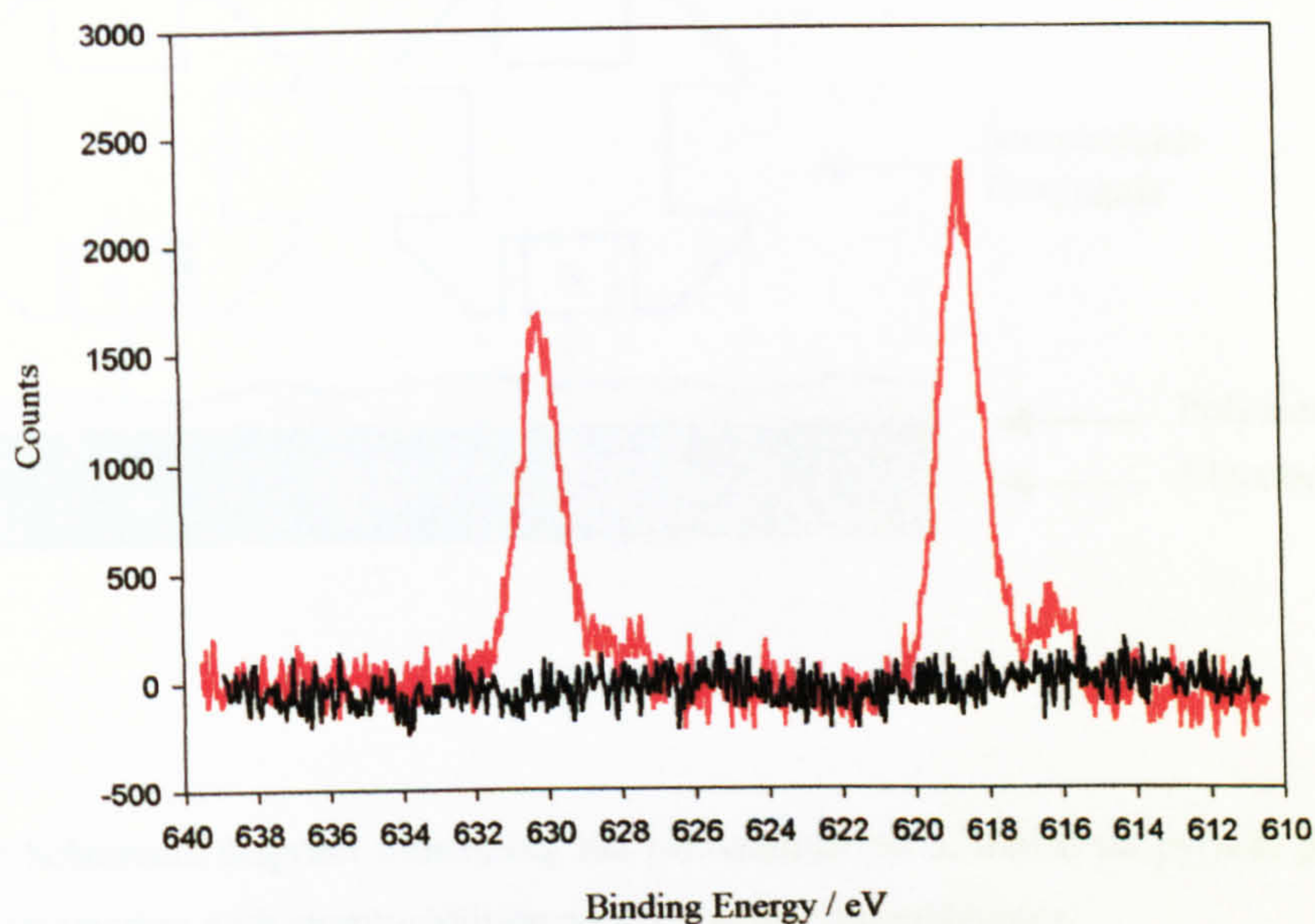
To confirm that the immobilisation was a consequence of the specific interaction of streptavidin with the biotinylated polymer, the biotin-streptavidin modified films were immersed in biotin solution. By doing so the solution based biotin was able to interact with the docking sites of streptavidin and slowly, through the nature of equilibrium processes, displace the bound streptavidin molecules from the polymer surface. This removal was apparent from analysis of the XPS data (Figure 7.5) as there was a reduction of iodine signal after immersion in biotin reduces. From XPS measurements it was noted that the reduction varied from 100% in some samples to and approximately 50 % in other samples.





**Figure 7.5:** S (2p) XPS spectra (TOA 90 degrees) of poly(PFP) after reaction with pentylamine biotin and DMSO for 15 minutes and C (1s) XPS spectra of poly(PFP) before (black) and after (red) reaction with pentylamine/ DMSO. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ .





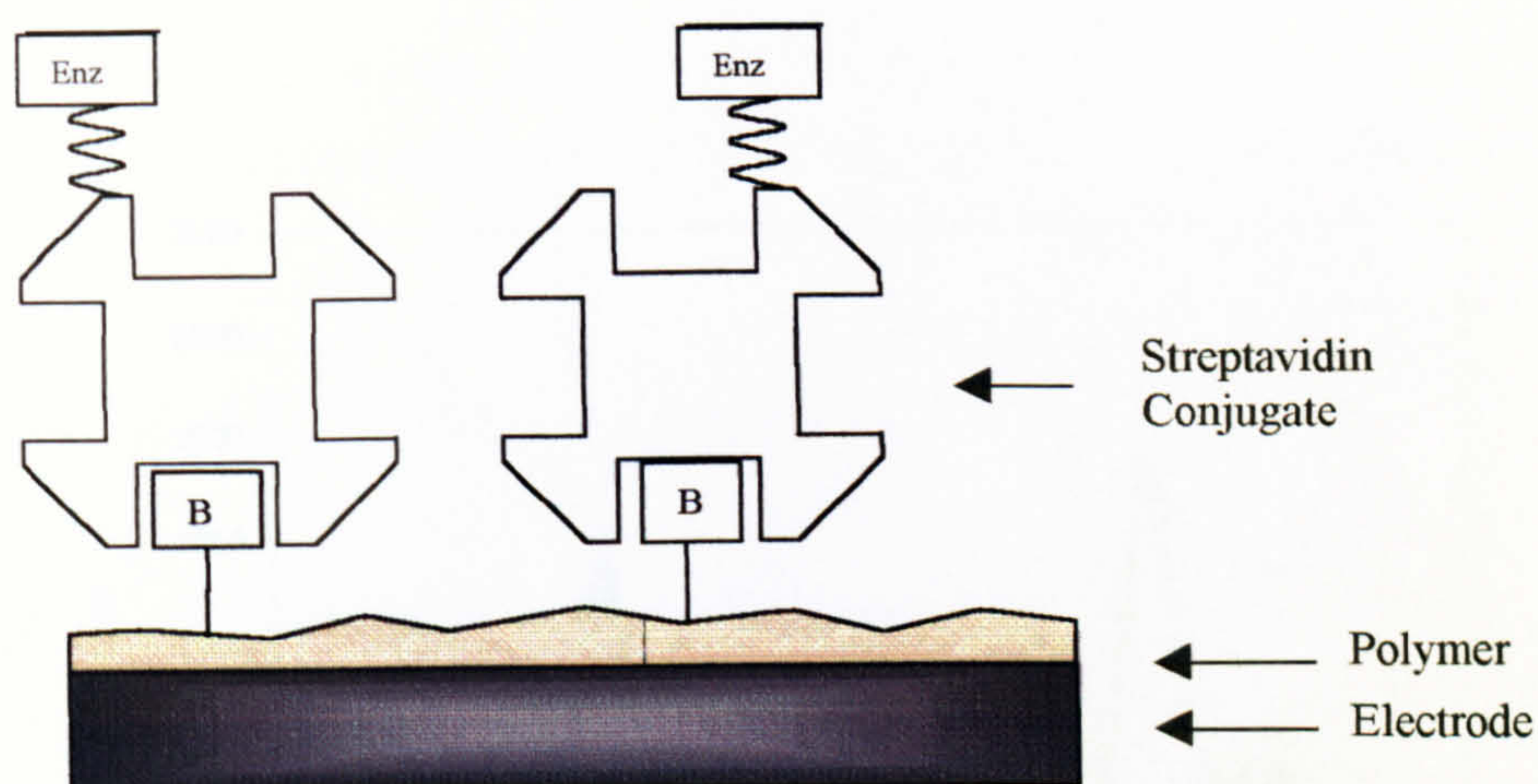
**Figure 7.5:** I (3d) XPS spectra (TOA 90 degrees) of pentylamine modified poly(PFP) after reaction with 0.1 mg/ml Iodine labelled Streptavidin and buffer solution (red) and subsequent reaction with biotin solution (black). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$  this was then reacted with pentylamine and DMSO solution.

#### 7.2.1.3 Reaction of Biotin Modified Polymer with Streptavidin Conjugates

The next stage of the development was to immobilise a streptavidin-enzyme conjugate on the biotin based polymers. For these experiments, it was decided to use streptavidin conjugated to hydrogen peroxidase (StHRP) and alkaline phosphatase (StAP) as both were commercially available (Figure 7.6).

The enzyme-streptavidin conjugates were also iodinated to allow easy detection using XPS. Figure 7.7 shows the XPS spectrum obtained after measurements were performed on the samples that were reacted with the streptavidin containing species. Iodine is present on the samples analysed, suggesting that immobilisation has occurred.





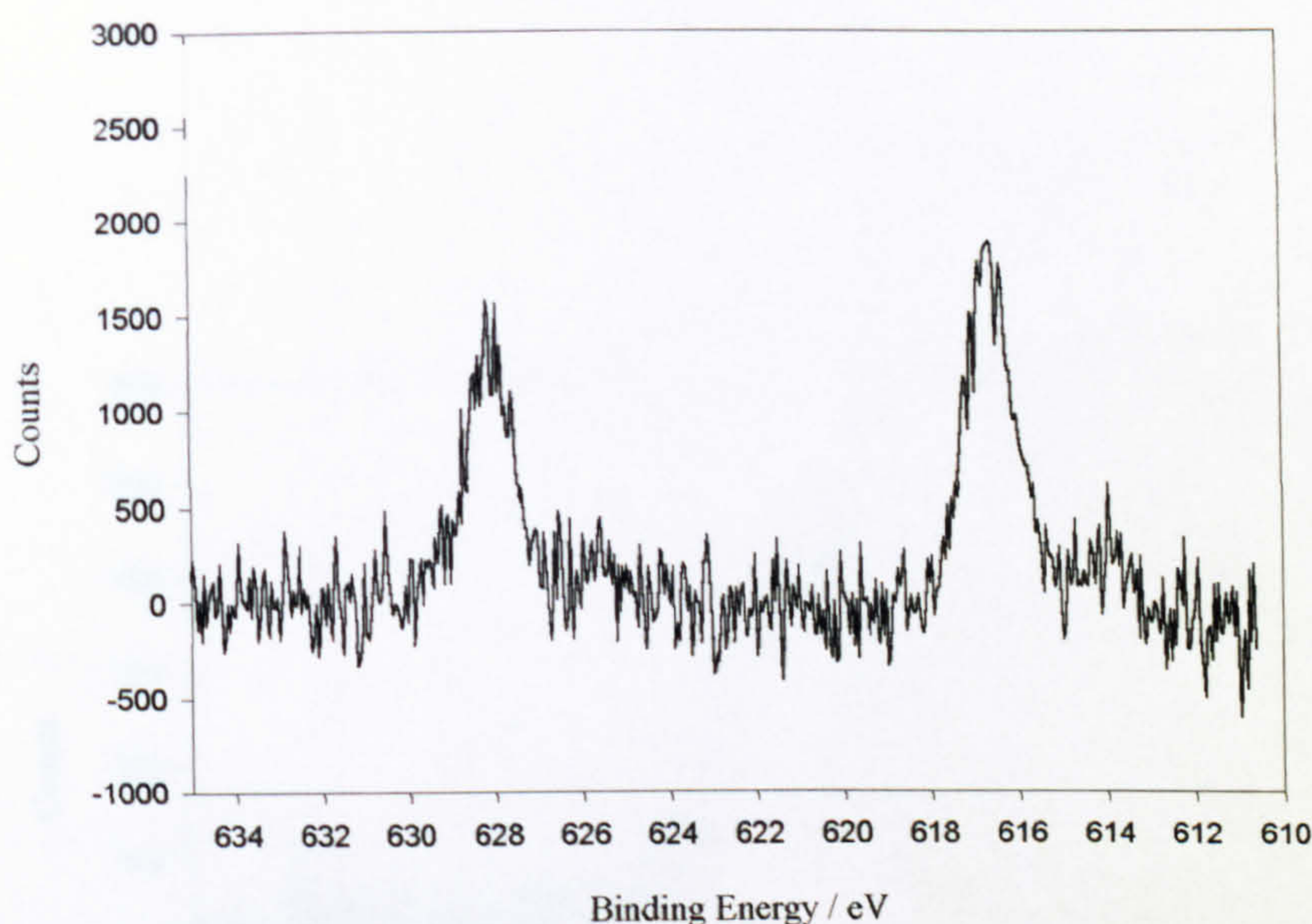
**Figure 7.6:** Schematic diagram illustrating the immobilisation of biotin on pyrrole polymer and the subsequent interaction with streptavidin-enzyme conjugate biomolecules.

To evaluate the extent of non-specific adsorption arising from the HRP (hydrogen peroxidase) or AP (alkaline phosphatase) moiety, competitive binding experiments using brominated HRP or AP were performed. For example, Figure 7.8 (a) shows the Br (3d) signal arising from the non-specific adsorption of brominated HRP on a biotinylated polymer film. When a similar film is immersed in a 50:50 solution of Iodo-streptavidin HRP and Br-HRP the size of the Br signal is reduced by 90%.

The lack of Br (3d) signal and the presence of I (3d) signal, both detected using XPS analysis, again showed that there was successful immobilisation of streptavidin-HRP conjugate. The biotin-streptavidin complex strategy, therefore, provides the basis of a useful system for the immobilisation of an enzyme at the biotin-modified poly(PFP) interface.

The control of nucleophilic reaction of the poly(PFP) film with pentylamine biotin proved difficult using the electrochemical method described, so it was decided to use an alternative approach for the immobilisation of biotin using a cystamine modified polymer, which can lead to the possibility of controlling the immobilisation of the streptavidin conjugated enzymes. This strategy is outlined in the following section.





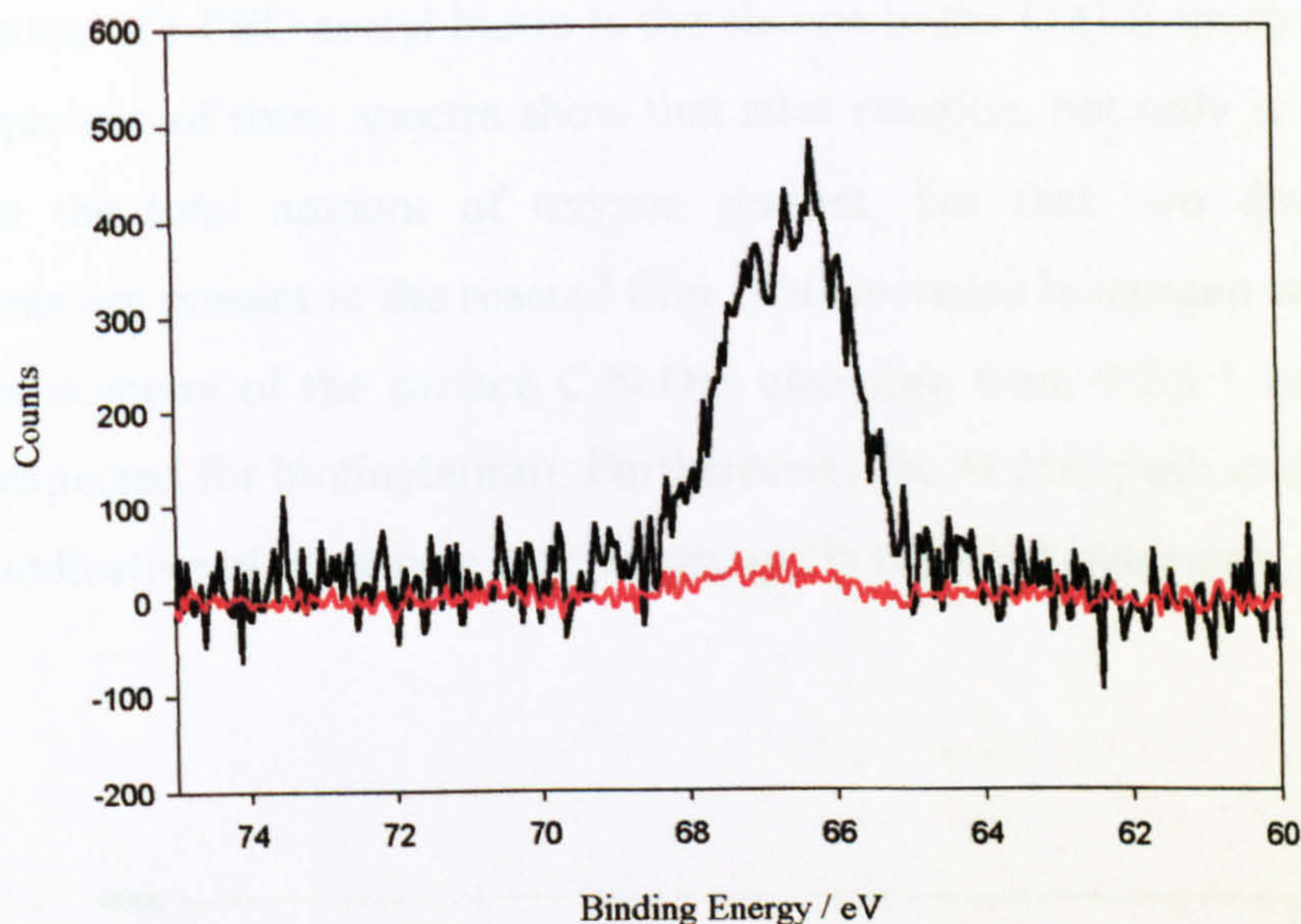
**Figure 7.7:** I (3d) XPS spectra (TOA 90 degrees) of pentylamine modified poly(PFP) after reaction with 0.1 mg/ml Iodine labelled Streptavidin-HRP and buffer solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup> this was then reacted with pentylamine and DMSO solution.

### 7.2.2 Reaction of Cystamine Modified Poly(PFP) with Biotin Derivative Followed by Immobilisation of Streptavidin and Streptavidin Conjugates

This technique involves the reaction of cystamine-modified poly(PFP) with an iodoacetyl biotin derivative and streptavidin respectively. As previously described, (Chapter 5) the reaction of the ester of poly(PFP) and cystamine produces a polymer, which has thiol groups available for nucleophilic reaction with another species. In the context of this study, the reaction investigated is with PEO-Iodoacetyl Biotin. PEO-Iodoacetyl Biotin, is a long chain, water-soluble compound that possesses a polyethylene glycol spacer arm. The PEO spacer arm imparts high water solubility to the reagent. The iodoacetyl functional group of the species reacts with free SH



groups at pH 7.5-8.5. The reaction occurs by nucleophilic substitution of iodine with a thiol group, resulting in a stable thioether bond. The reaction of PEO-Iodoacetyl Biotin with  $-SH$  groups present is rapid (if the reaction is carried out at a pH between 7.5-8.5).



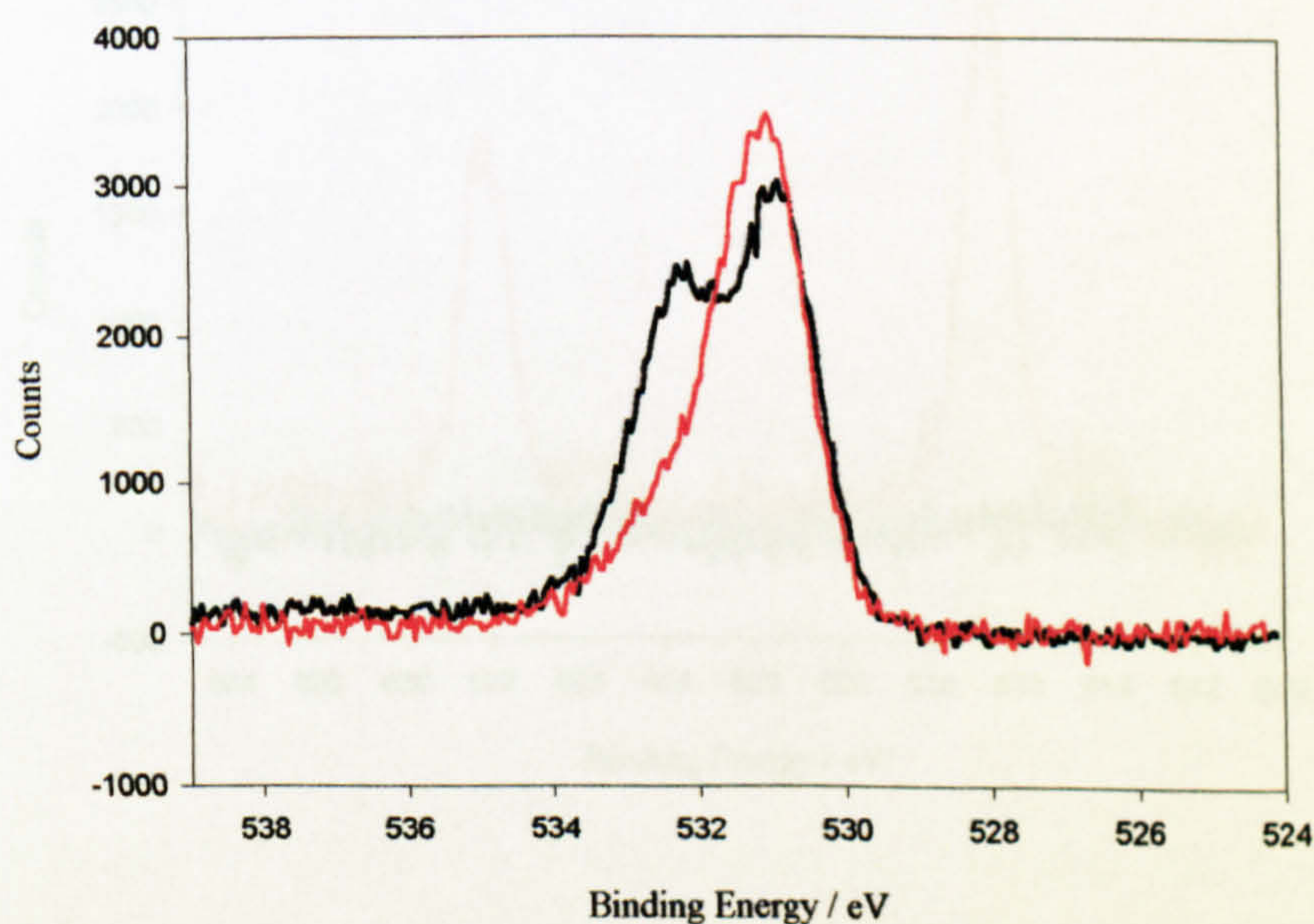
**Figure 7.8:** Br (3d) XPS spectra of poly(PFP) (black) and pentylamine biotin modified poly(PFP) (red) after reaction with 0.1 mg/ml bromine labelled HRP for 1 hour. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . To produce the modified film poly(PFP) was reacted with pentylamine and DMSO solution.

An important advantage of using this system for the immobilisation of streptavidin-enzyme conjugate is that cystamine reaction with poly(PFP) can be electrochemically controlled (see Chapter 5, Section 5.2.3). This can allow the possibility of the patterning of different enzymes on electrodes of the same device.



### 7.2.2.1 Reaction of Cystamine Modified Poly(PFP) Film with I-PEO-acetylbiotin

Cystamine modified polymers were prepared as discussed earlier in Chapter 5. The next stage of the formation of the polymeric multicomponent polymer is the immobilisation of biotin containing species. An indication of the subsequent immobilisation of I-PEO acetyl biotin is the change in the O (1s) spectrum of Figure 7.10. Comparison of these spectra show that after reaction, not only is there a small increase in the total amount of oxygen present, but that two distinct oxygen environments are present in the reacted film. This increase in oxygen was quantified by the stoichiometry of the surface C:N:O:S changing from 9:2:1:1 to 27:6:6:2 (as would be expected for biotinylation). Furthermore, the O (1s) peak at approximately 531 eV is indicative of ether type oxygen groups in the PEO spacerarm.

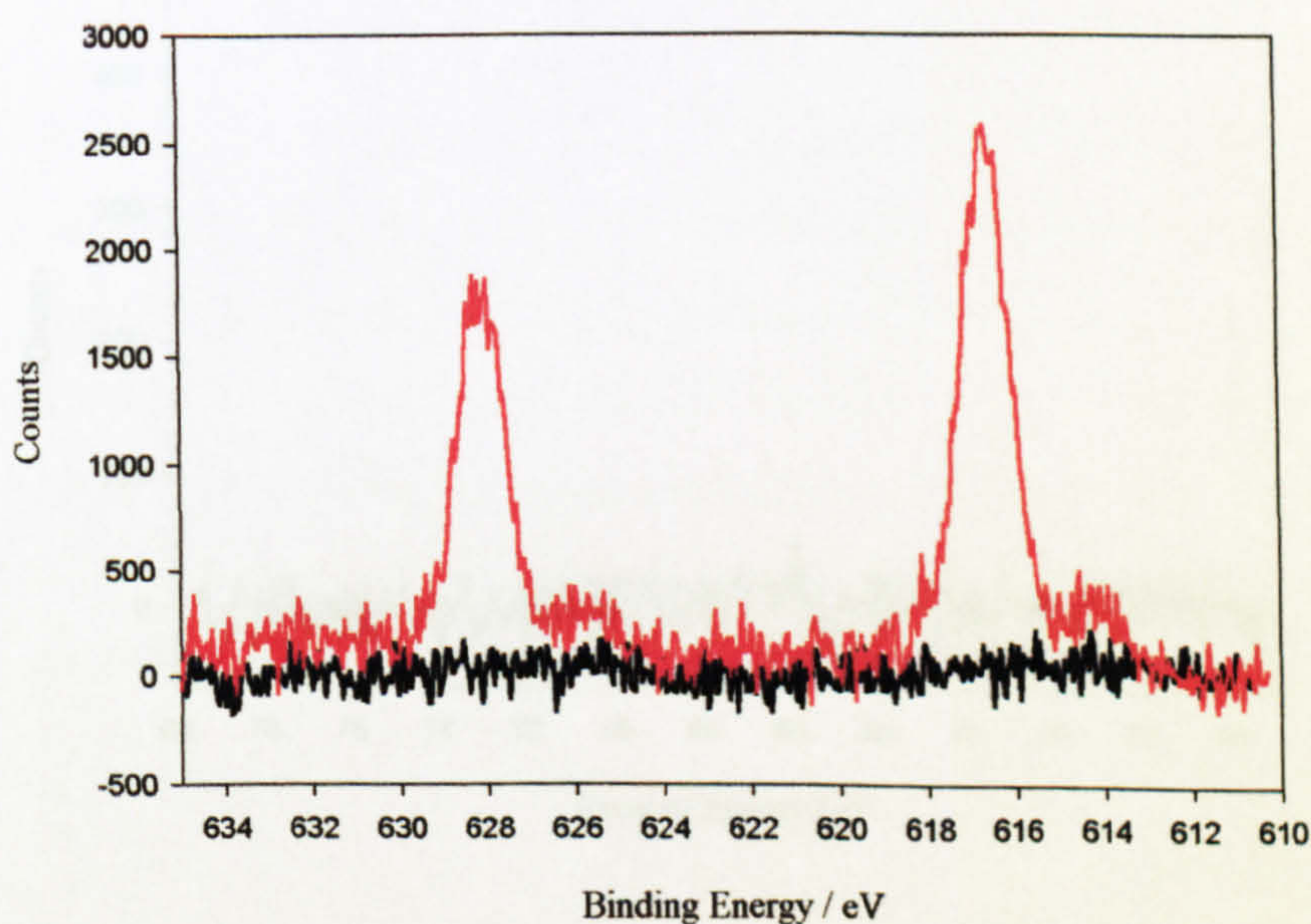


**Figure 7.10:** O (1s) XPS spectra (TOA 90 degrees) of cystamine modified poly(PFP) film before (red) and after reaction (black) with I PEO Biotin/ buffer solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>. This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.



### 7.2.2.2 Reaction of Modified Film with Streptavidin Conjugates

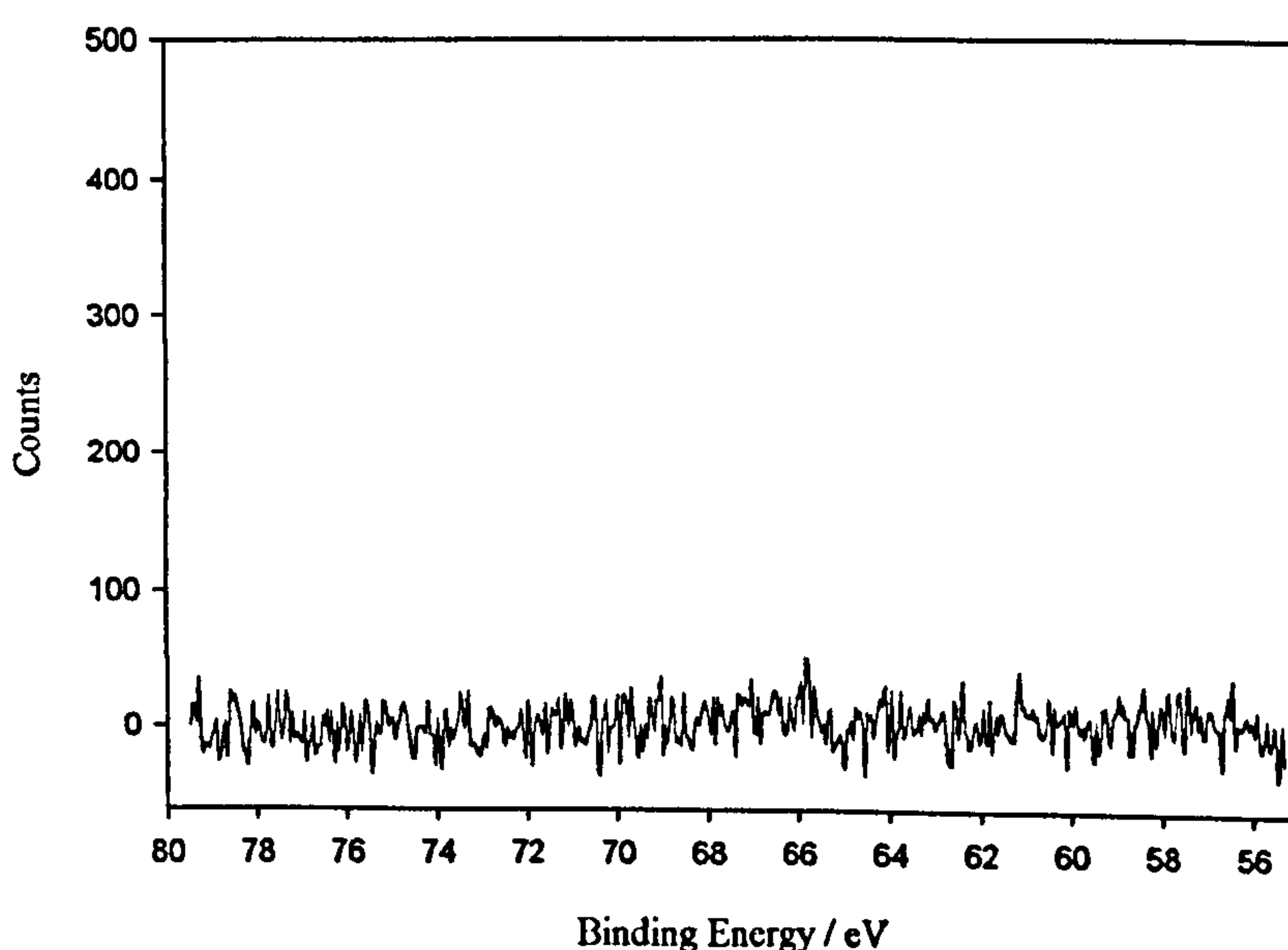
The attachment of this streptavidin functionality allows reaction of the available biotin with streptavidin and streptavidin-enzyme conjugates. Using the same labelled streptavidin molecules as those used in the previous section, experiments were performed to investigate the immobilisation of streptavidin on the cystamine-biotin modified films. The presence of I (3d) in the spectra seen in Figure 7.11, show that the immobilisation of reactive species with streptavidin present was successful. Again, in competitive adsorption experiments in which brominated enzymes were not conjugated to streptavidin were used there was no Br (3d) signal (Figure 7.12) detected using XPS and, therefore, unsuccessful immobilisation. This demonstrated that HRP immobilisation is via the streptavidin moiety.



**Figure 7.11:** I (3d) XPS spectra of cystamine modified poly(PFP) after reaction with aqueous I-PEO Biotin (black) and then subsequent reaction with iodinated streptavidin HRP (red). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.



Comparisons were also made of the two different immobilisation strategies that lead to the formation of biotin-streptavidin complexes. Both the biotin-modified polymer and cystamine-biotin modified polymers were reacted with  $0.1 \text{ ml}^{-1}$  iodinated streptavidin for one hour. The results obtained from these investigations suggested that there was an increase in the amount of streptavidin immobilised on the cystamine-modified polymer as there was an increase in iodine content seen after XPS analysis (Figure 7.13). The cause for this increase may have been due to the spatial arrangement of the biotin as a consequence of the PEO spacer arm present within the cystamine-biotin modified polymer compared to the more densely packed biotin of the biotin modified polymer.



**Figure 7.12:** Br (3d) XPS spectra of cystamine modified poly(PFP) after reaction with brominated HRP. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.

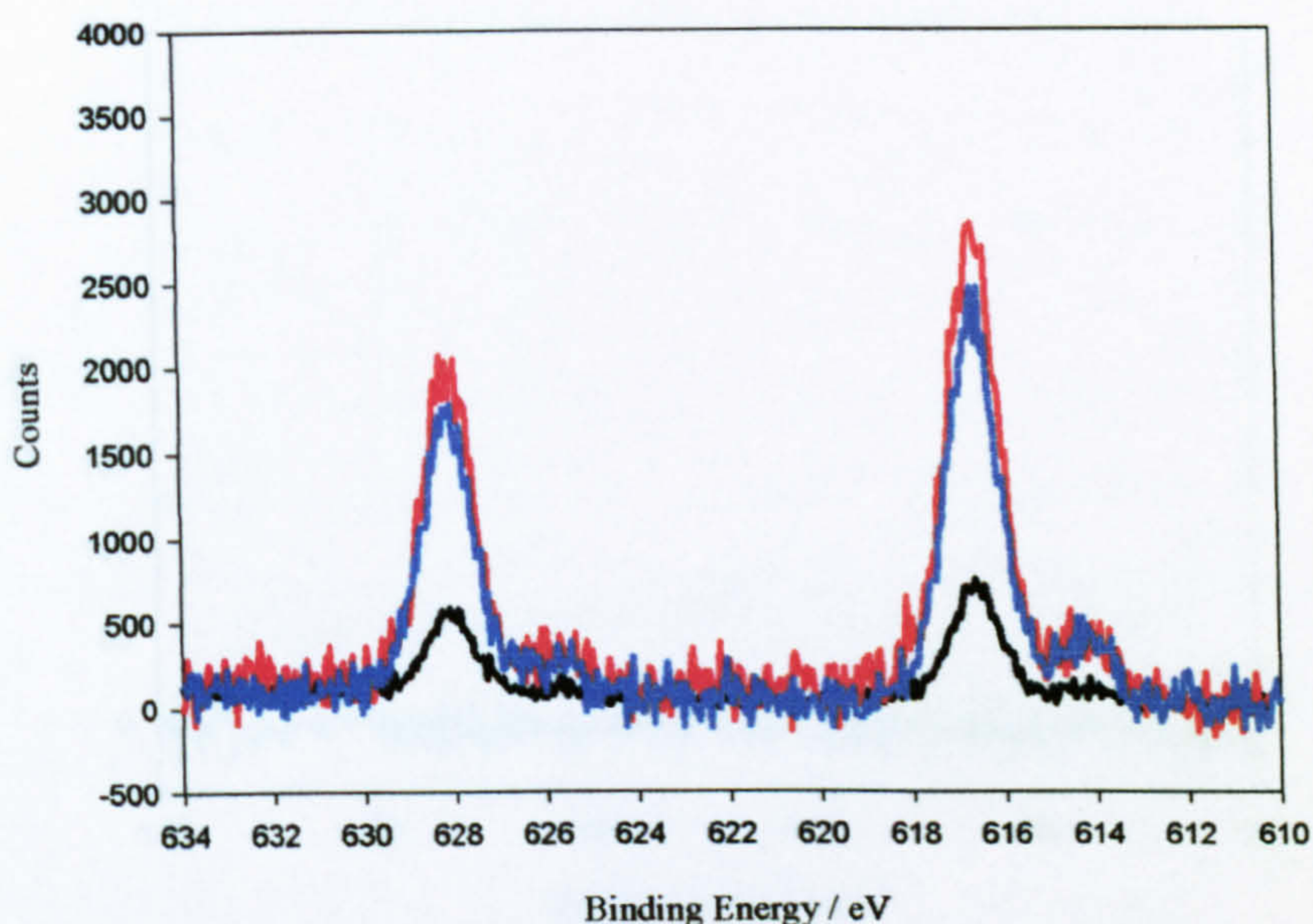


### **7.2.3 Reaction Control of Poly(PFP) with Cystamine Prior to Biotin-Streptavidin Modification**

The method of forming a cystamine-biotin-streptavidin template and utilising the possibility of inhibition of immobilisation of cystamine provides the basis for the selective patterning of streptavidin-enzyme conjugates on different electrodes of the same device. Initial investigations were undertaken to ascertain if the functionalisation of the PFP polymer using Iodo-PEO acetylbiotin could be controlled by the presence of cystamine on the surface of the polymer film. To this end, it was important to determine that the presence of thiol groups on the polymer film was necessary for the nucleophilic reaction with Iodo-PEO acetylbiotin (as described in section 7.2.2.1). In addition, it was important to ensure that immobilisation of streptavidin does not occur when the biotin derivative was not present on the surface of the polymer film.

XPS examination of the S (2p), O (1s) and I (3d) regions was used to probe whether iodo acetyl activated biotin either non specifically adsorbed or reacted with a poly(PFP) film that had been previously oxidised. The absence of S (2p) and I (3d) signal (Figure 7.14 and 7.15). Furthermore, Figure 7.16 shows the O (1s) spectra of doped poly(PFP) after reaction with cystamine and Iodo-PEO acetylbiotin and indicates that there was no significant increase in oxygen content after this reaction indicating there was an absence of the biotin derivative on the polymer surface.

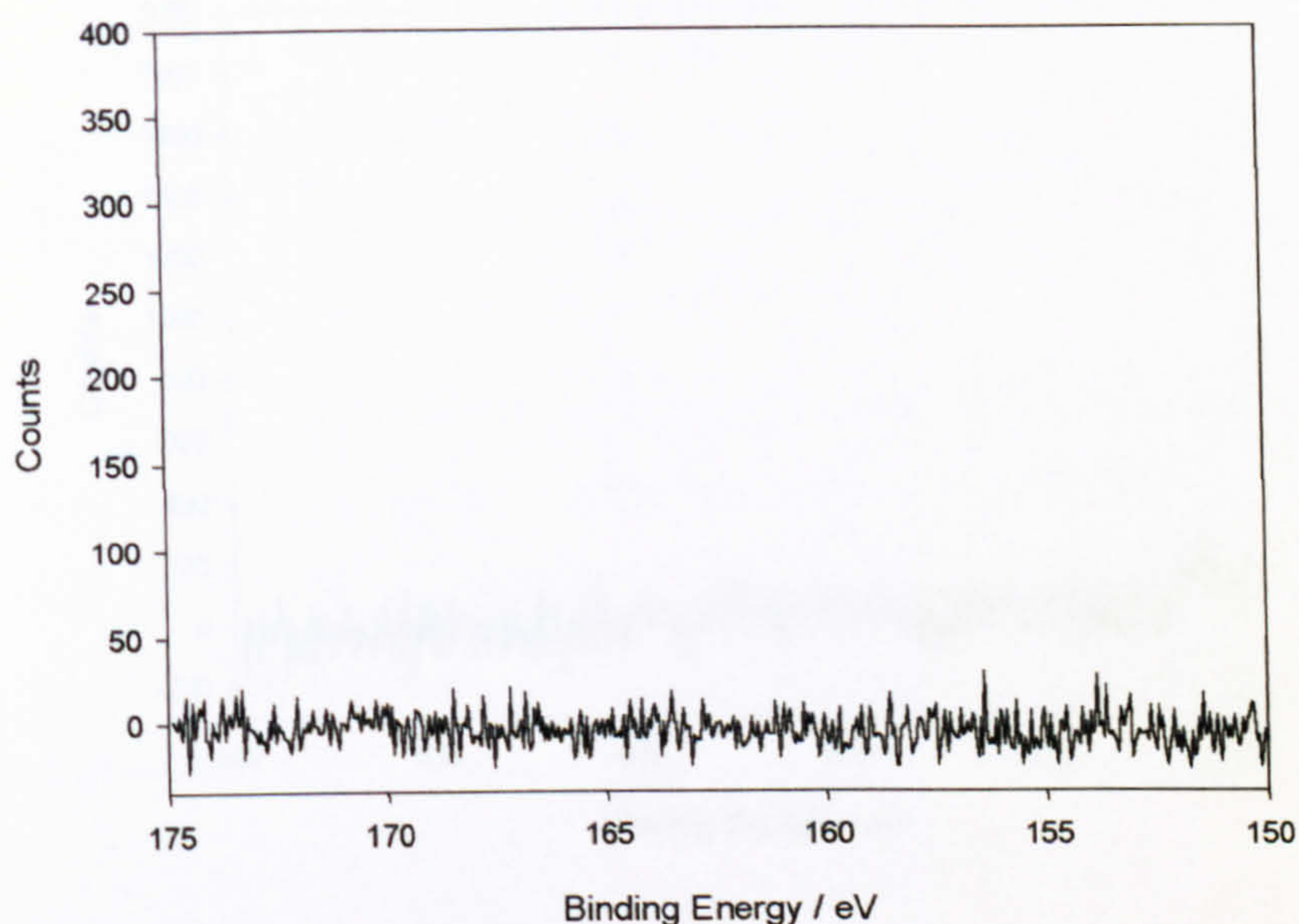




**Figure 7.13:** I (3d) XPS spectra of poly(PFP) (black), biotin modified poly(PFP) (blue) and cystamine-biotin modified poly(PFP) (red) after reaction with iodinated streptavidin HRP. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>. The poly(PFP) was then reacted with 1 mM cystamine and DMSO solution followed by reaction with aqueous I-PEO Biotin solution to produce the cystamine-biotin modified poly(PFP) film. To produce the biotin modified film, poly(PFP) was reacted with pentylamine and DMSO solution.

Further investigations show that there was adsorption of iodinated streptavidin on poly(PFP) as there was an appearance of a significant doublet seen between 615 and 630 eV. It was found that non-specific adsorption of streptavidin on poly(PFP) decreased significantly as the immersion time was reduced as seen in Figure 7.17. However, when the reaction time of streptavidin with a cystamine/biotin-modified polymer was reduced from 1 hour to 0.5 hours the amount of streptavidin immobilisation was not significantly affected. Thus if a protocol for sequential immobilisation is to be developed, the kinetics of the non-specific and specific immobilisation should be studied further.





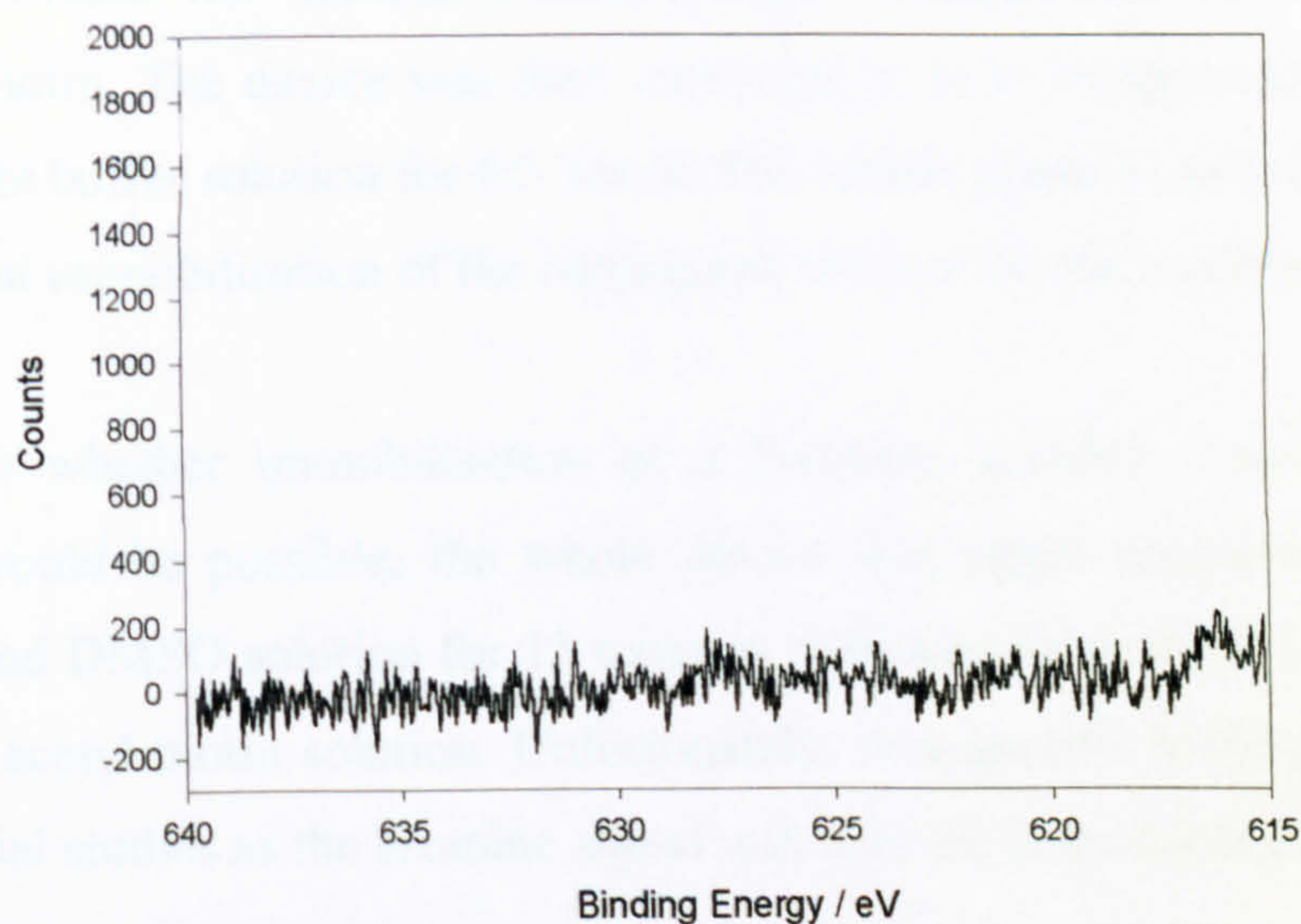
**Figure 7.14:** S (2p) XPS spectra of poly(PFP) after reaction with Iodo-PEO acetylbiotin/ buffer solution for 15 minutes. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ .

#### 7.2 4 Selective Patterning of Biotin-Streptavidin Modified Poly(PFP)

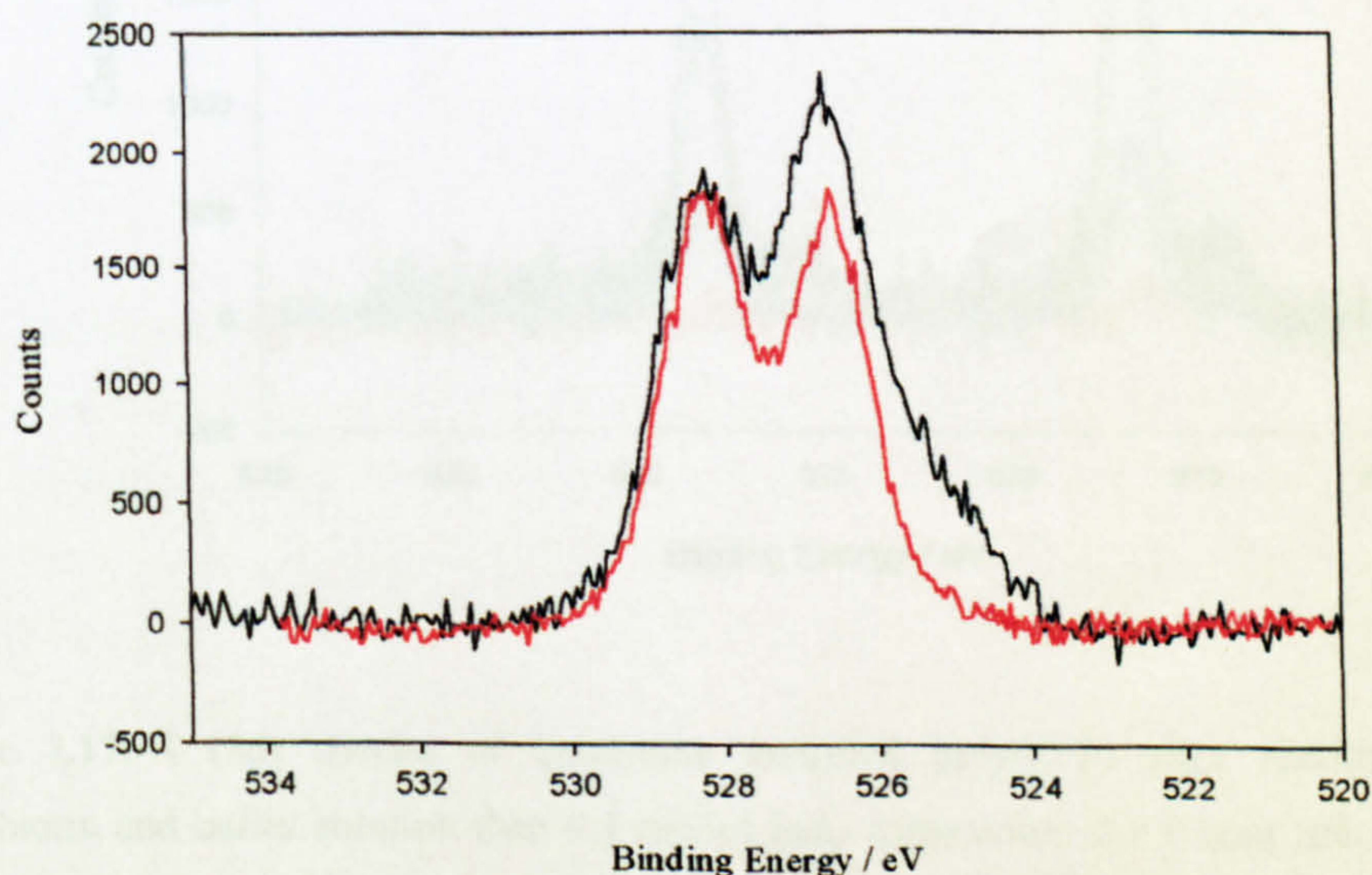
To investigate the applicability of this method for the selective patterning of streptavidin-enzyme conjugates on a device, poly(PFP) was electrodeposited onto the electrodes of a two-electrode device. As before the thickness of the poly(PFP) was controlled to prevent bridging of polymer between the two electrodes.

Following is an explanation of each individual step for the preparation of the device, which is used to demonstrate the selective patterning of streptavidin-enzyme conjugate. In each section the XPS images, which were obtained during the experiment will be shown. After each stage of the experiment the device was washed with the suitable solution, dried and prepared for analysis using the XPS Imaging facility. The main regions of interest were S (2p) and I (3d).





**Figure 7.15:** I (3d) XPS spectra of poly(PFP) after reaction with Iodo-PEO acetylbiotin/ buffer solution for 15 minutes. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ .

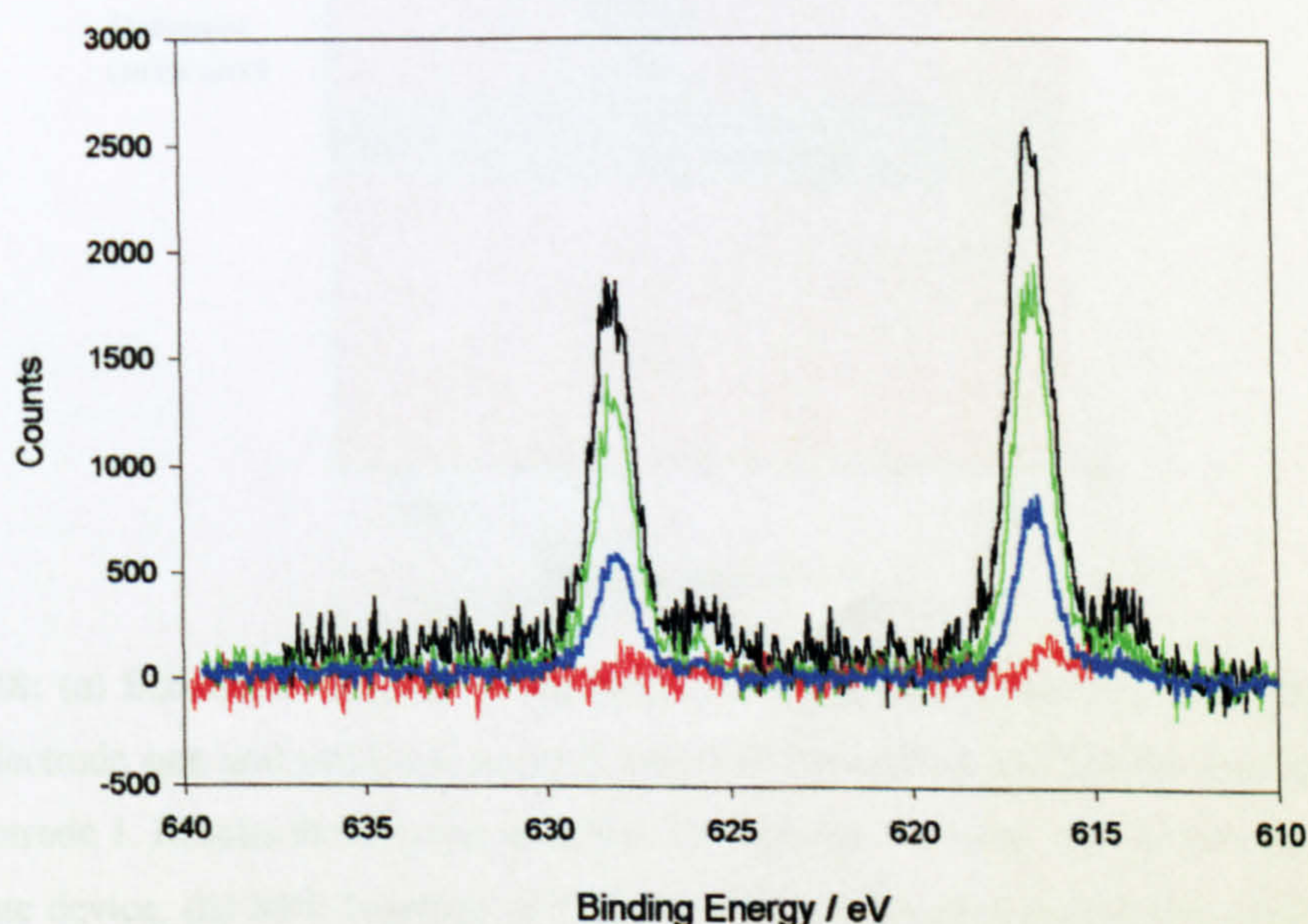


**Figure 7.16:** O (1s) XPS spectra of unreacted poly(PFP) (red) and doped poly(PFP) after reaction with cystamine solution followed by immersion in Iodo-PEO acetyl biotin solution (black). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ .



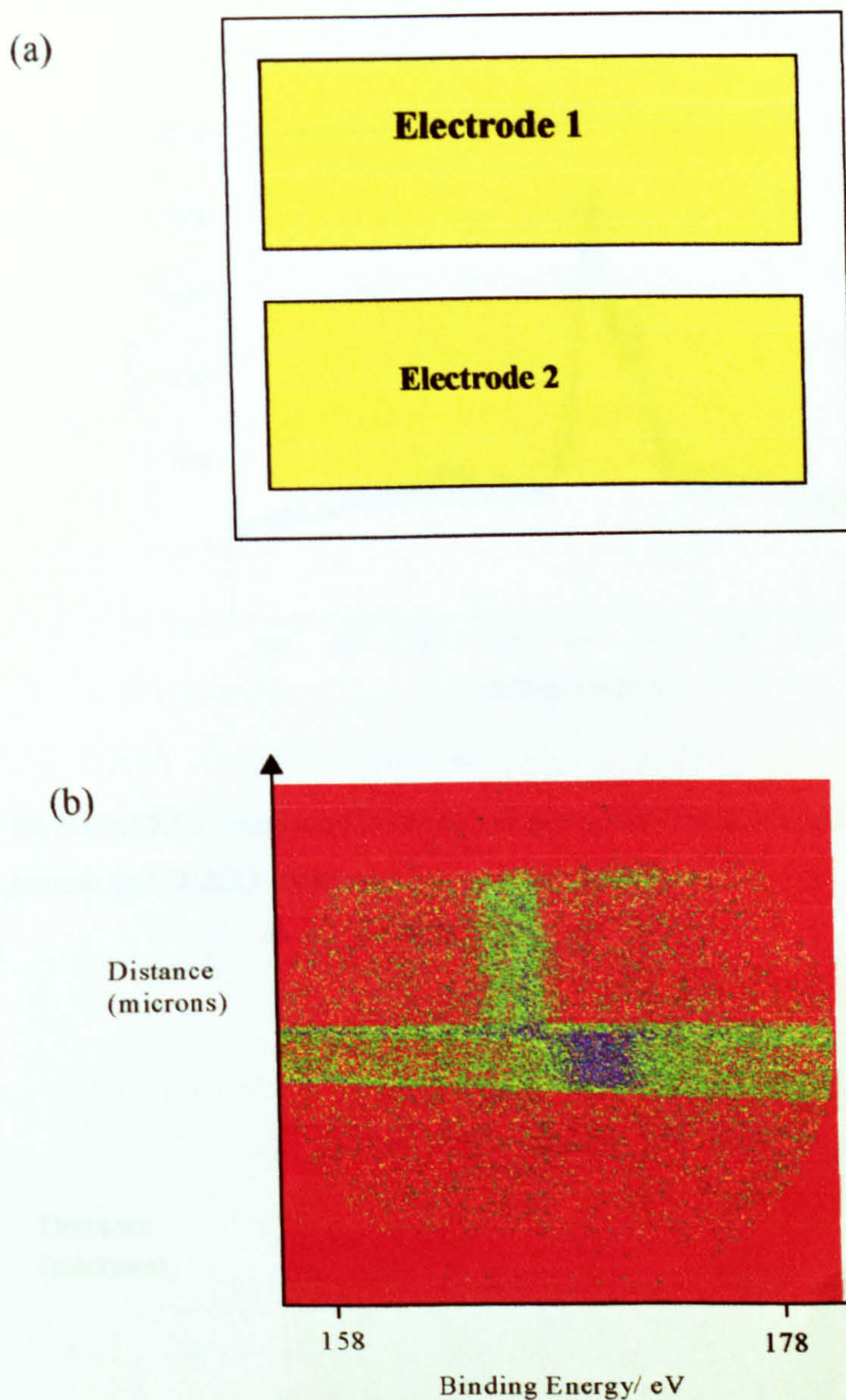
A cystamine modified device was immersed in a 10 mM Iodo-PEO acetylbiotin and pH 8 phosphate buffer solution for 1 hour. The immobilisation of cystamine on one electrode provides the necessary thiol groups needed to react with the PEO-iodoacetyl biotin. The device was then immersed in Iodo-streptavidin-HRP and pH 7.4 phosphate buffer solution for 0.5 hours. The iodine signal is now apparent due to the successful immobilisation of the conjugated enzyme on the modified film.

To establish whether immobilisation of a bromine labelled streptavidin-enzyme conjugate would be possible, the whole device was again immersed in the 1mM cystamine and DMSO solution for 15 minutes, followed by an hour reaction with 10 mM I-PEO acetyl biotin solution. Unfortunately, non-specific binding was apparent in these initial studies as the bromine signal was seen on both electrodes.



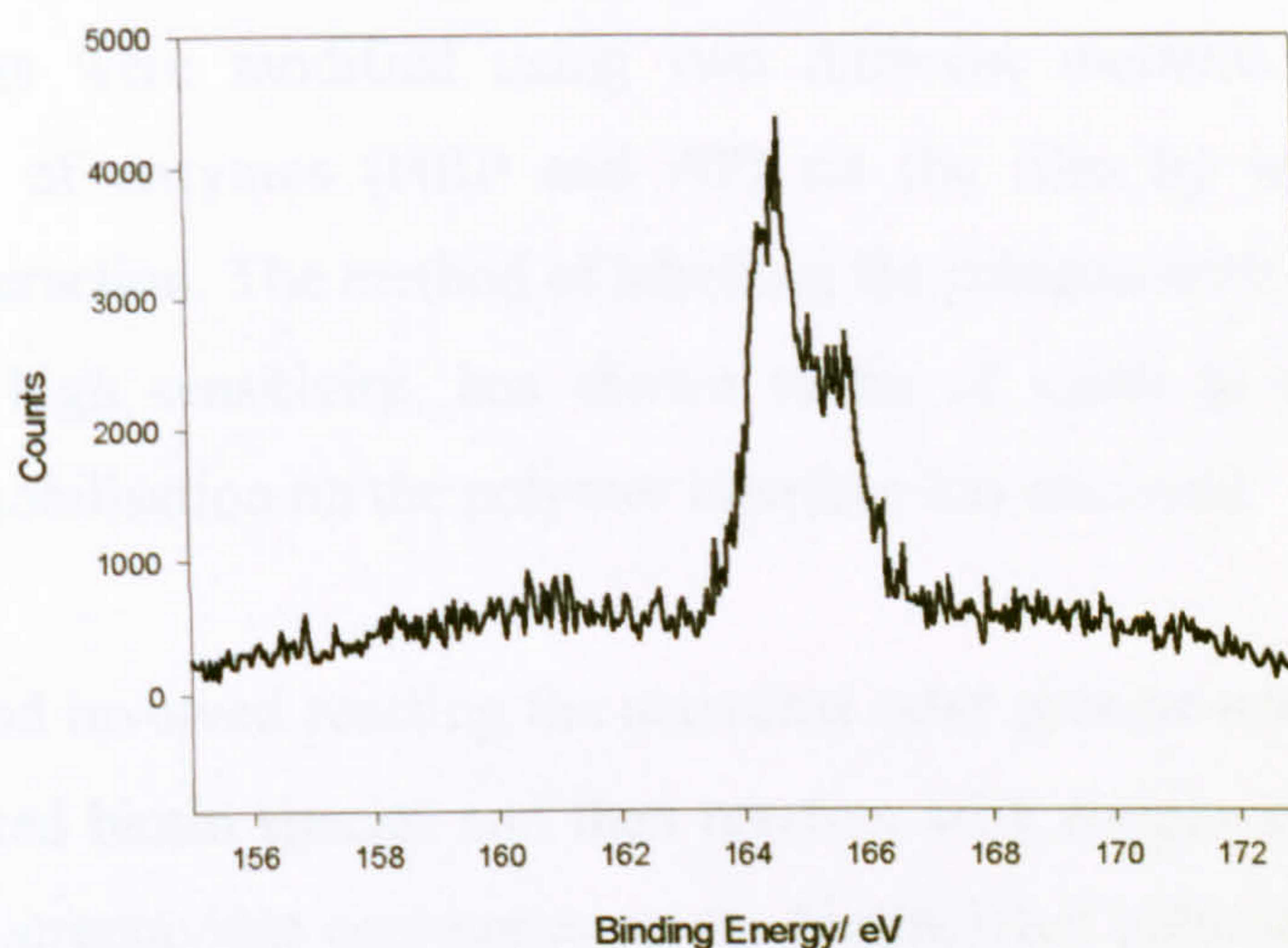
**Figure 7.17:** I (3d) spectra of cystamine modified poly(PFP) after reaction with Iodo-PEO acetylbiotin and buffer solution then 0.1 mg/ml Iodo streptavidin for 1 hour (black), poly(PFP) film after reaction with Iodo streptavidin for 1 hour (green) and after 0.5 hours (blue) and cystamine modified poly(PFP) after reaction with Iodo-PEO acetylbiotin for 15 minutes (red). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . This was then reacted with 1 mM cystamine and DMSO solution to produce the cystamine modified poly(PFP) film.



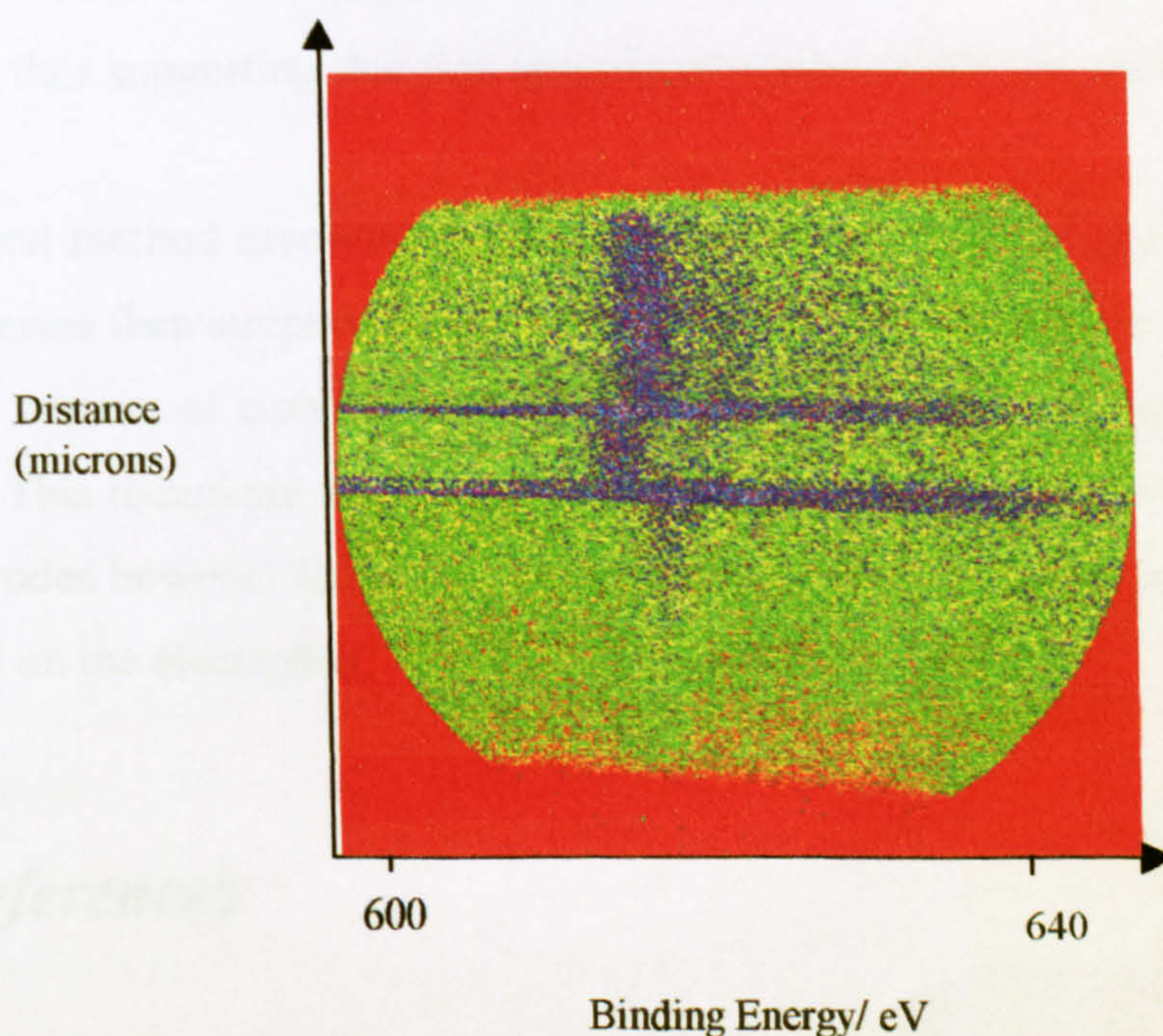


**Figure 7.18:** (a) Schematic diagram of the structure of the device used in these selective patterning studies. Electrode one and electrode two are poly(PFP) modified and further modification is carried out on electrode 1. Results demonstrated on figures 7.18 (b), 7.19 and 7.20 are of subsequent reactions on the same device. (b) XPS Imaging of S (2p) region of two digit poly(PFP) modified device after reaction with 1mM cystamine and DMSO solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and electrode two was doped before immersing into the cystamine solution. It is apparent that the electrode one has cystamine present due to the strong signal in the sulphur region. The signal that can be seen in the middle of the device is due to the underlying glass.





**Figure 7.19:** S (2p) XPS spectrum (TOA 90°) of poly(PFP) modified electrode one after reaction with 1 mM cystamine and DMSO solution



**Figure 7.20:** XPS Imaging of I (3d) region of two digit electrode device after reaction with aqueous I-PEO acetylbiotin and then subsequent reaction with iodinated streptavidin HRP. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and one electrode was reacted with 1 mM cystamine and DMSO solution. It is apparent that the electrode one (see Figure 7.18) has been modified due to the strong signal in the iodine region. The signal that can be seen in the middle of the device is due to the underlying glass.



### 7.3 Summary

Poly(PFP) films were modified using two different methods that leads to the immobilisation of enzymes (HRP and AP) on the film by utilising the biotin-streptavidin interaction. The method of labelling the proteins with unique XPS labels, which have a high sensitivity, has shown to be of value in evaluating whether successful immobilisation on the polymer interface has occurred.

The first method involved reacting the activated ester present within poly(PFP) with amine terminated biotin species and then reacting with streptavidin conjugates. The binding of the streptavidin conjugates on the biotinylated polymers was then probed using XPS. These studies have indicated that when using conjugated enzymes the entire bound enzyme is present through the streptavidin interaction with the substrate. When non-conjugated enzymes were used there was visible XPS signal detected, thus suggesting that non specific adsorption does not occur.

The second method involves reacting the cystamine modified poly(PFP) film with a biotin species then streptavidin-enzyme conjugate. This technique appeared to lead to a greater amount of enzyme immobilised on the surface compared with the previous method. This technique also provides the opportunity to selectively pattern enzymes on electrodes however initial experiments were unsuccessful as non-specific binding occurred on the electrodes.

### 7.4 References

1. Kittigul, L., Suthachana, S., Kittigul, C., Pengruangrojanachai, V., "Immunoglobulin M-capture biotin-streptavidin enzyme-linked immunosorbent assay for detection of antibodies to dengue viruses", *The American Society of Tropical Medicine and Hygiene*, vol. 59, no. 3, pp. 352, 1998.
2. Schetters, H., "Avidin and streptavidin in clinical diagnostics", *Biomolecular Engineering*, vol. 16, pp. 73, 1999.



## 7.3 Summary

Poly(PFP) films were modified using two different methods that leads to the immobilisation of enzymes (HRP and AP) on the film by utilising the biotin-streptavidin interaction. The method of labelling the proteins with unique XPS labels, which have a high sensitivity, has shown to be of value in evaluating whether successful immobilisation on the polymer interface has occurred.

The first method involved reacting the activated ester present within poly(PFP) with amine terminated biotin species and then reacting with streptavidin conjugates. The binding of the streptavidin conjugates on the biotinylated polymers was then probed using XPS. These studies have indicated that when using conjugated enzymes the entire bound enzyme is present through the streptavidin interaction with the substrate. When non-conjugated enzymes were used there was visible XPS signal detected, thus suggesting that non specific adsorption does not occur.

The second method involves reacting the cystamine modified poly(PFP) film with a biotin species then streptavidin-enzyme conjugate. This technique appeared to lead to a greater amount of enzyme immobilised on the surface compared with the previous method. This technique also provides the opportunity to selectively pattern enzymes on electrodes however initial experiments were unsuccessful as non-specific binding occurred on the electrodes.

## 7.4 References

1. Kittigul, L., Suthachana, S., Kittigul, C., Pengruangrojanachai, V., "Immunoglobulin M-capture biotin-streptavidin enzyme-linked immunosorbent assay for detection of antibodies to dengue viruses", *The American Society of Tropical Medicine and Hygiene*, vol. 59, no. 3, pp. 352, 1998.
2. Schetters, H., "Avidin and streptavidin in clinical diagnostics", *Biomolecular Engineering*, vol. 16, pp. 73, 1999.



3. Ijiro, K., Ringsdorf, H., "Protein-DNA Double and Triple Layers: Interaction of Biotinylated DNA Fragments with Solid Supported Streptavidin Layers", *Langmuir*, vol. 14, no. 10, pp. 2796, 1998.
4. Niemeyer, C. M., Ceyhan, B., Blohm, D., "Functionalization of Covalent DNA-Streptavidin Conjugates by means of biotinylated modulator components", *Bioconjugate Chemistry*, vol. 10, no. 5, pp. 708, 1999.
5. Chen, Q., Kobayashi, Y., Takeshita, H., Hoshi, T., Anzai, J., "Avidin-Biotin System-Based Enzyme Multilayer Membranes for Biosensor Applications: Optimization of Loading Choline esterase and Choline Oxidase in the Bienzyme Membrane for Acetylcholine Biosensors", *Electroanalysis*, vol. 10, pp. 94, 1998.
6. Cosnier, S., Stoytcheva, M., Senillou, A., Perrot, H., Furriel, R. P. M., Leone, F. A., "A biotinylated conducting polypyrrole for spatially controlled construction of an amperometric biosensor", *Analytical Chemistry*, vol. 71, pp. 3692, 1999.
7. Cosnier, S., Lepellec, A., "Poly(pyrrole-biotin): a new polymer for biomolecule grafting on electrode surfaces", *Analytical Chemistry*, vol. 44, pp. 1833, 1999.
8. Cosnier, S., Galland, B., Gondran, C., Le Pellec, A., "Electrogeneration of biotinylated functionalized polypyrroles for simple immobilisation of enzymes", *Electroanalysis*, vol. 10, pp. 808, 1998.
9. Torres-Rodriguez, L. M., Billon, M., Roget, A., Bidan, G., "A polypyrrole-biotin based biosensor: elaboration and characterization", *Synthetic Metals*, vol. 102, pp. 1328, 1999.
10. Fraker, P. J., Speck, J. C., "Mediator for protein iodination", *Biochemical and Biophysical Research Communication*, vol. 80, pp. 849, 1978.



# Chapter 8

## *Non-specific Binding of Modified Poly(PFP) Films*

### **8.0 Introduction**

Non-specific binding (NSB) is the undesirable immobilisation/ adsorption of biomolecules on the surface of the polymer-modified electrode. In the context of this study, this may include an unwanted side reactions with the activated ester of the poly(PFP) film or, alternatively, adsorption onto the polymer surface. By changing the functional groups present within the polymer backbone the degree of unwanted adsorption of biomolecules onto the polymer interface may alter.

Investigations exploring polyethylene glycol (PEG) as a fouling-resistant material have been performed by others<sup>1-5</sup>. Investigations have found that bacteria and biomaterial do not adsorb well to surfaces composed of PEG-modified polymers.<sup>7-9</sup> The basis of the results described in this chapter has led to the formation of a technique to prevent non-specific binding onto polymer based interfaces. This involves modifying the polymer with polypropylene glycol (PPG) and determining whether NSB can be prevented on the modified polymer.

Covalent attachment of PPG to poly(PFP) involved reaction of the terminal group of a PPG derivative to the surface of the polymer. Again the aminolysis reaction was utilised and involved a poly(PFP) film reacting with amine terminated PPG species. As part of this investigation, PPG of varying molecular weights were used to determine whether a change in molecular weight has an affect on the degree of NSB.



Section 8.2.1 describes the reaction of poly(PFP) with PPG derivatives, and is followed by results achieved from NSB investigations using the modified polymer. These investigations compare the NSB at three types of polymer interface: poly(PFP), hydrolysed poly(PFP) and PPG modified poly(PFP). The XPS facility was used as a suitable method to detect the presence of these labelled biomolecules on the polymer-solution interface. In these studies fluorescence is not a suitable form of detection as the polymer film have background fluorescence and the gold substrate causes high reflectivity.

## ***8.1 Materials***

**Solvents:** DMSO and MeCN were obtained from Aldrich.

**TEAP** was prepared as described in Chapter 2.

**Bis-(2-aminopropyl) propylene glycol (PPG)** with formula weight of 130 gmol<sup>-1</sup> (PPG-130), PPG with formula weight of 800 gmol<sup>-1</sup> (PPG-800) and PPG with formula weight of 1900 gmol<sup>-1</sup> (PPG-1900) were obtained from Pierce.

**Labelled Biomolecules** prepared as described in Chapter 7.

## ***8.2 Results and Discussion***

### **8.2.1 Immobilisation of PPG on Poly(PFP) Film**

The reaction of the amine terminated PPG chains with the activated ester again involves the nucleophilic substitution of the pentafluorophenyl leaving group producing amide bonds. The poly(PFP) film was reacted with the three different formula weights; PPG-130, PPG-800 and PPG-1900, all in DMSO.

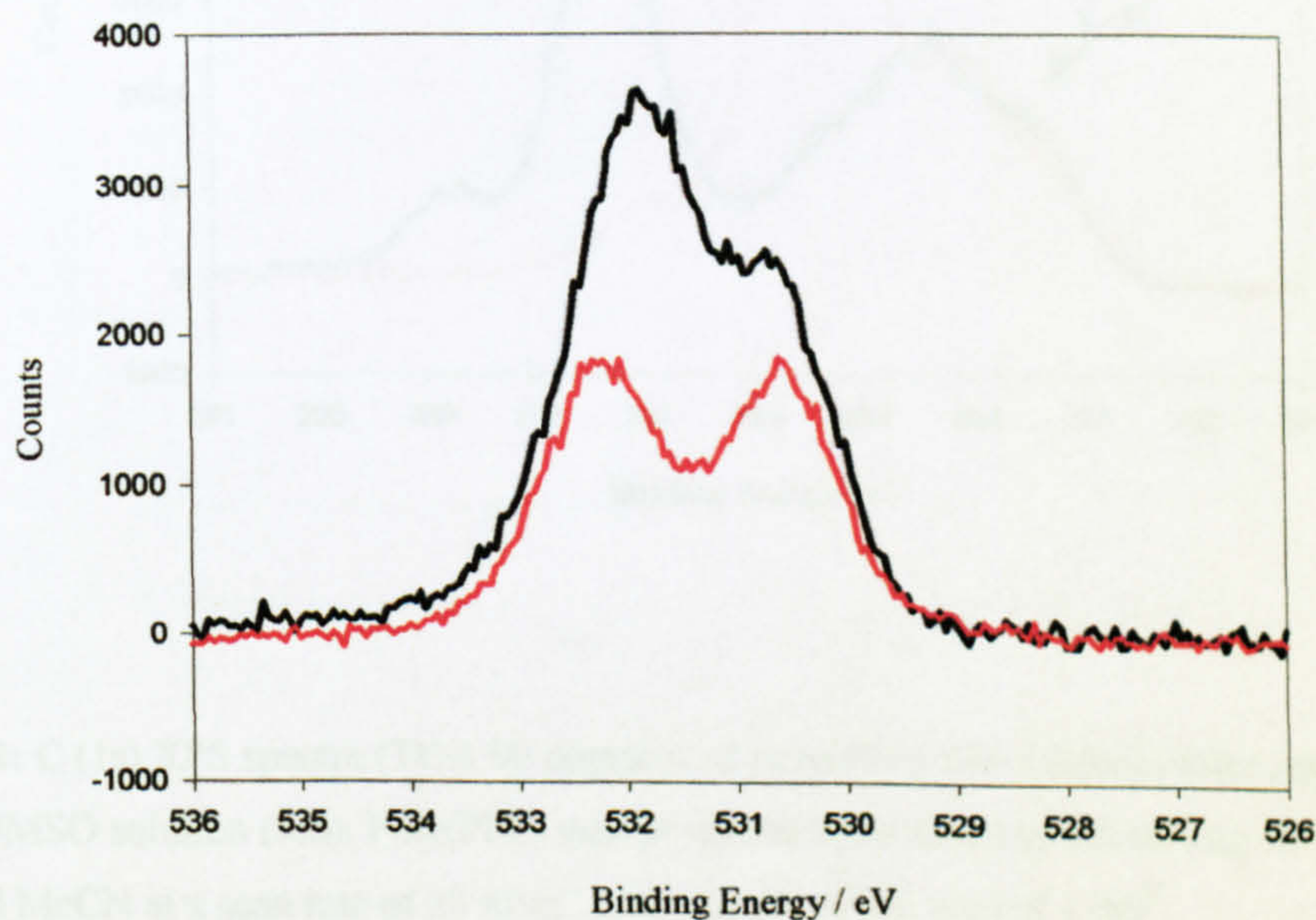
PPG has a high oxygen and CH<sub>2</sub> group content, therefore, detection was made possible by using XPS C (1s) and O (1s) spectra. As seen in Figure 8.1 a change in oxygen signal was apparent corresponding to the modification of the poly(PFP) with



PPG. There were two oxygen species present within a PPG modified poly(PFP) film. The first comes from the oxygen of the carbonyl present within the amide group and the second from the ether linkages of the PPG itself.

In addition, there was an increase in C-H peak in C (1s) spectra due to increased presence of C-O from the attached PPG chain (Figure 8.2).

Again the formation of the amide band during immobilisation was detected using FTIR (Figure 8.3), where the presence of amide I and II bands are seen at 1540 and 1680  $\text{cm}^{-1}$ .

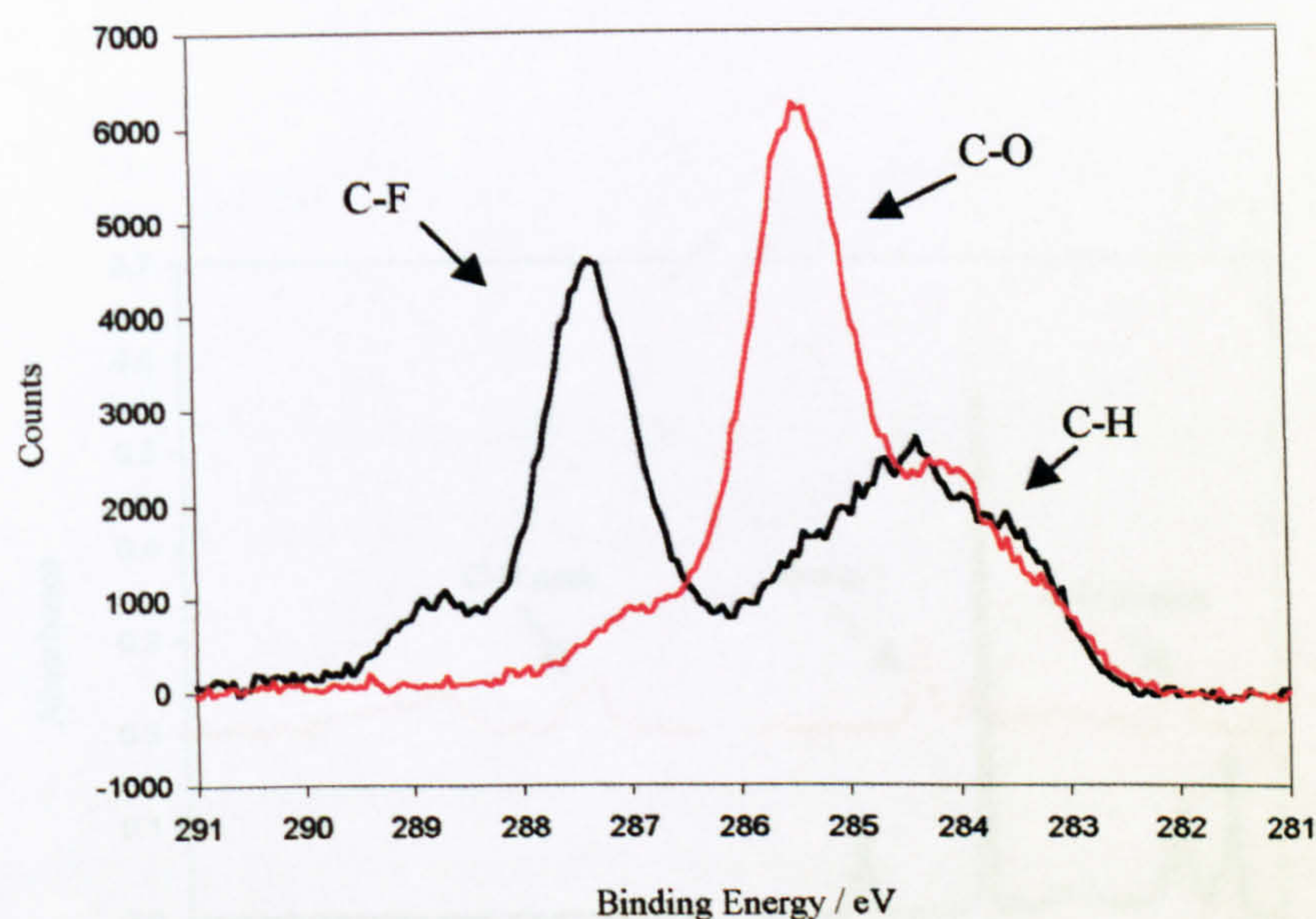


**Figure 8.1:** O (1s) XPS spectra (TOA 90 degrees) of poly(PFP) films before (red) and after reaction with 1 mM PPG-800 and DMSO solution (black). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20  $\text{mVs}^{-1}$  and with electrode area of 1  $\text{cm}^2$ .



### 8.2.2 Non-specific Binding Studies

PPG chains tethered on a surface exhibit the ability to sterically exclude macromolecules and particles (steric stabilisation). These characteristics are particularly useful for preventing the adsorption of proteins.<sup>1</sup>

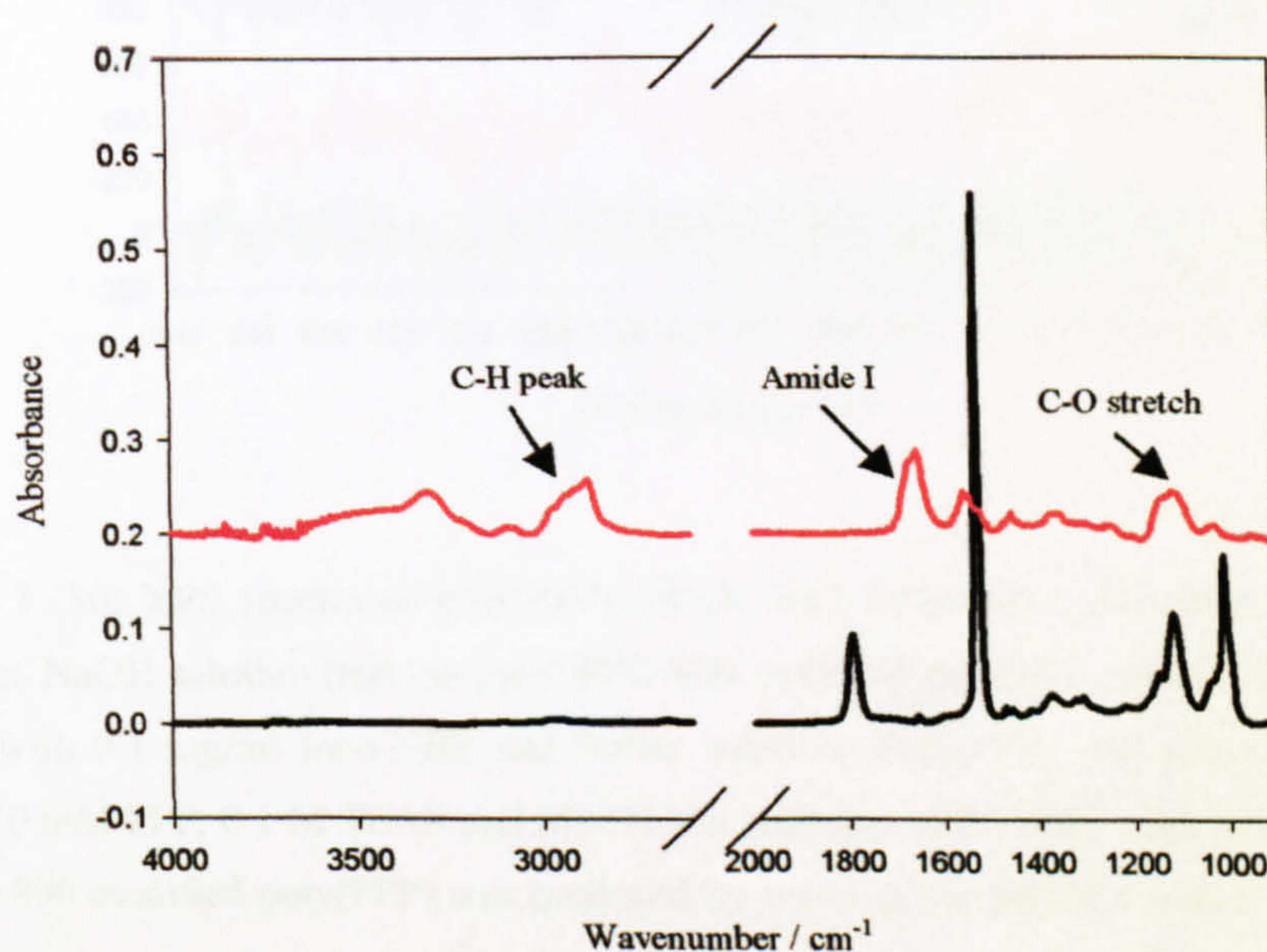


**Figure 8.2:** C (1s) XPS spectra (TOA 90 degrees) of poly(PFP) films (black) after reaction with PPG-800 and DMSO solution (red). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>.

To determine the degree of NSB that occurs on poly(PFP) films, the unreacted polymer coated electrodes were immersed in various solutions containing iodine labelled biomolecules and were subsequently analysed using XPS to detect the presence of iodine doublet. To illustrate NSB the unreacted poly(PFP) films were immersed in Iodo- HRP, Iodo-BSA, Iodo-cytochrome c and Iodo-streptavidin solutions. Figures 8.4, 8.5 and 8.6 illustrate XPS spectra of polymers after incubation with these biomolecules and show iodine signals were visible due to the adsorption



of these biomolecules on the polymer-solution interface of the poly(PFP) films. It was observed that changing incubation conditions could affect the degree of NSB of the biomaterial on the polymer surface. For example, an increase in time the polymer was immersed in the solution can cause an increase in signal detected, therefore, it was important to keep the incubation time constant for each experiment. In addition, an increase in the concentration of biomolecule within the buffer solution can increase the amount of biomolecule, which adsorbs on the polymer surface thus solution concentrations were kept constant for the different experiments performed.

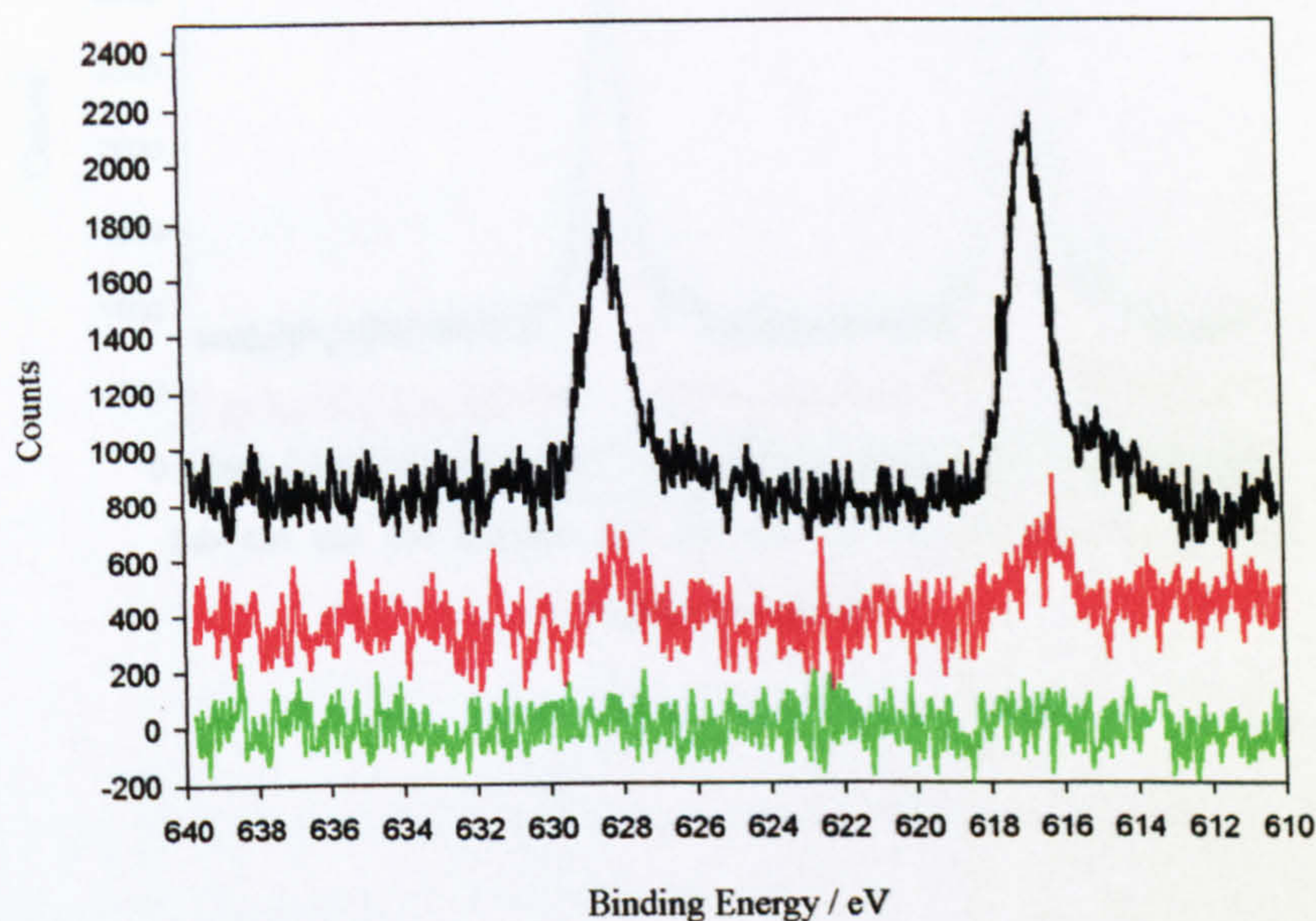


**Figure 8.3:** FTIR spectra of unreacted poly(PFP) film (black) and homopolymer film after reaction with amine terminated PPG-800 and DMSO solution (red). Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>.

To establish whether the prevention or decrease in adsorption would occur by simply using hydrolysed poly(PFP) films, similar experiments were performed. Immersing the films in a semi-aqueous sodium hydroxide solution produced these hydrolysed polymers. Hydrolysis was confirmed by detecting the COO<sup>-</sup> stretch at 1580 cm<sup>-1</sup>



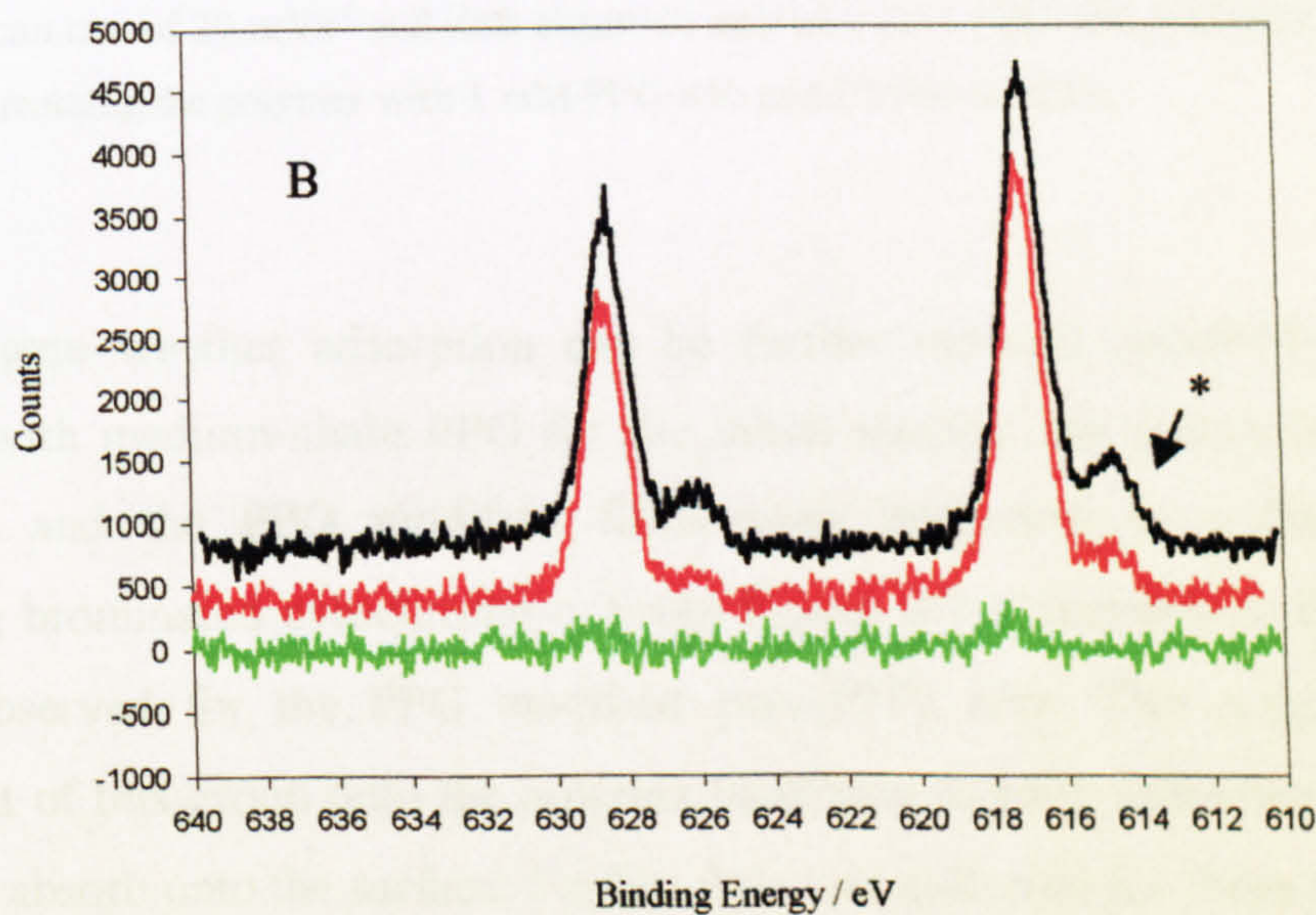
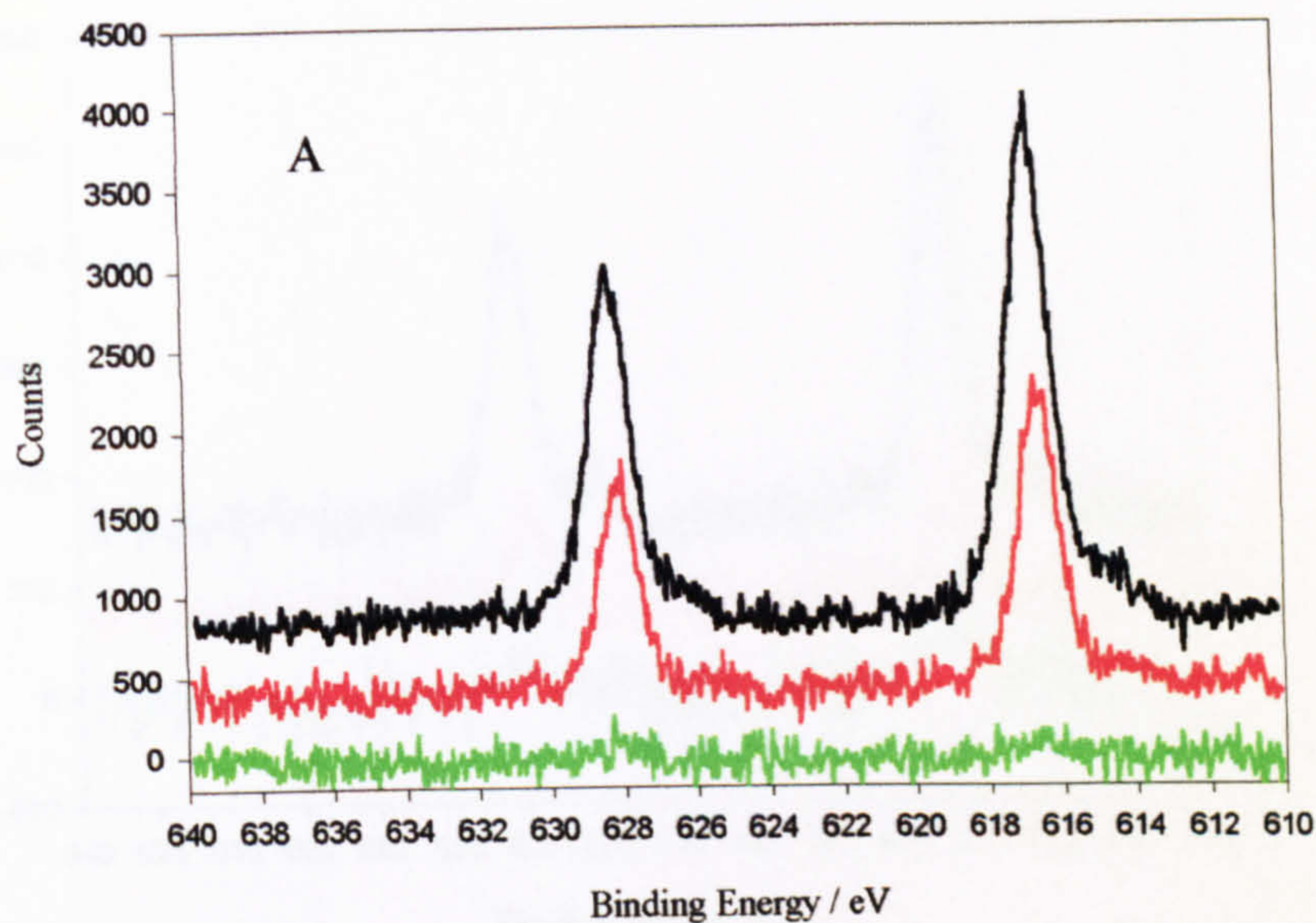
using RAIRS. The samples were then immersed in the Iodo-HRP, Iodo-BSA, Iodo-cytochrome c and Iodo-streptavidin solutions and were analysed using XPS (Figures 8.4, 8.5 and 8.6).



**Figure 8.4:** I (3d) XPS spectra of poly(PFP) (black line), hydrolysed poly(PFP) by reacting with semi-aqueous NaOH solution (red line) and PPG-800 modified poly(PFP) (green line) after reaction for 1 hour with 0.1 mg/ml Iodo-HRP and buffer solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . PPG-800 modified poly(PFP) was produced by reacting the polymer with 1 mM PPG-800 and DMSO solution.

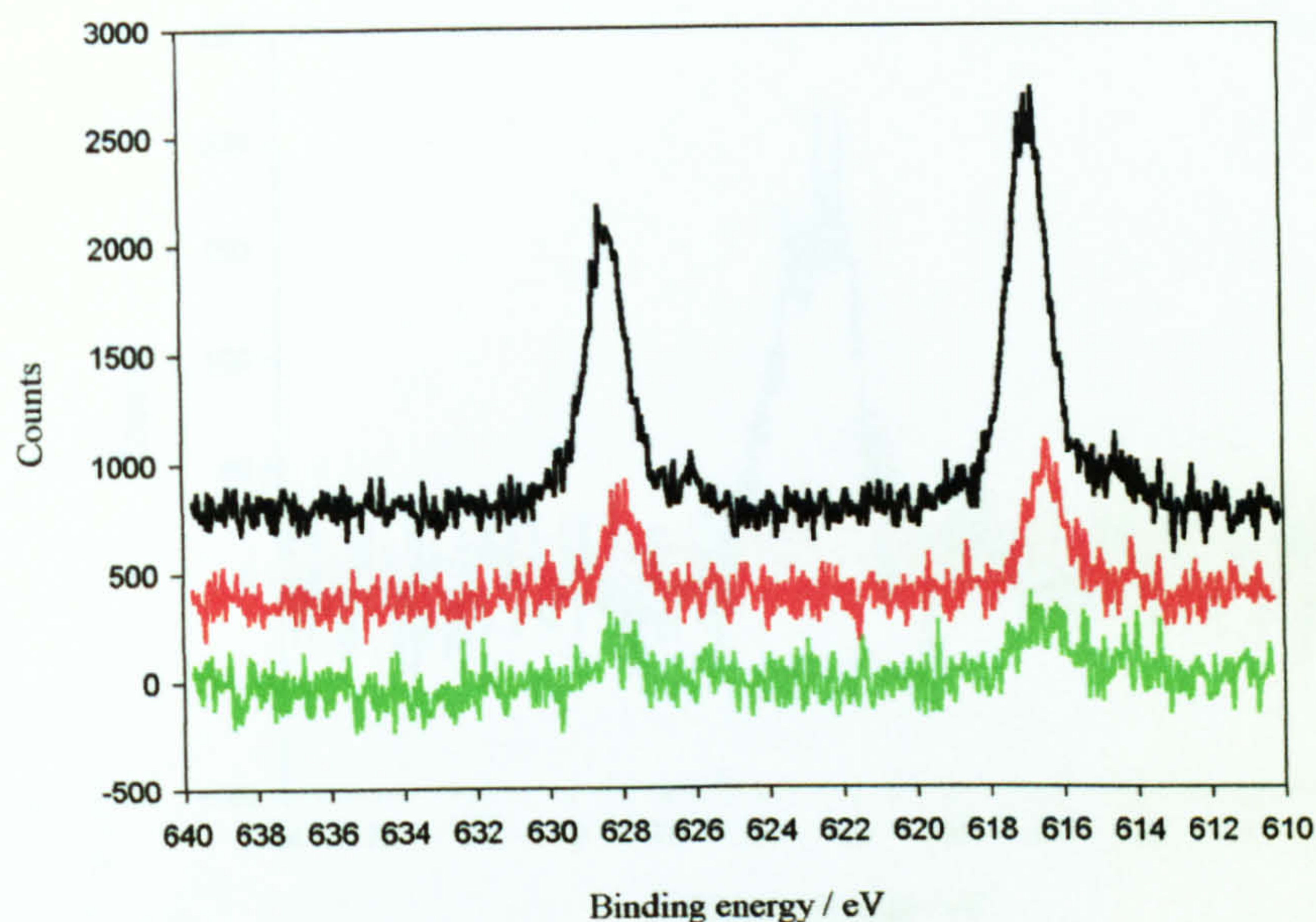
It was observed whilst comparing XPS spectra obtained after reaction of hydrolysed poly(PFP) film and poly(PFP) film that there was a change in the amount of material absorbed on the surface. The hydrolysis of the PFP group changes the chemical structure of the polymeric matrix and may, consequently, affect the interaction with biomolecules. It can be seen that there was a decrease in iodine signal when the hydrolysed films were used, therefore, suggesting a decrease in adsorption.





**Figure 8.5:** I (3d) XPS spectra of poly(PFP) (black line), hydrolysed poly(PFP) (red line) and PPG-800 modified poly(PFP) (green line) after reaction for 1 hour in (A) 0.8 mg/ml Iodo-BSA and buffer solution and (B) 0.1 mg/ml Iodo-cytochrome c and buffer solution. PPG-800 modified poly(PFP) was produced by reacting the polymer with 1 mM PPG-800 and DMSO solution. Note the presence of the additional peak (\*) as indicated in (B), this is due to the presence of  $\Gamma^-$  species occasionally formed after extended x-ray exposure.

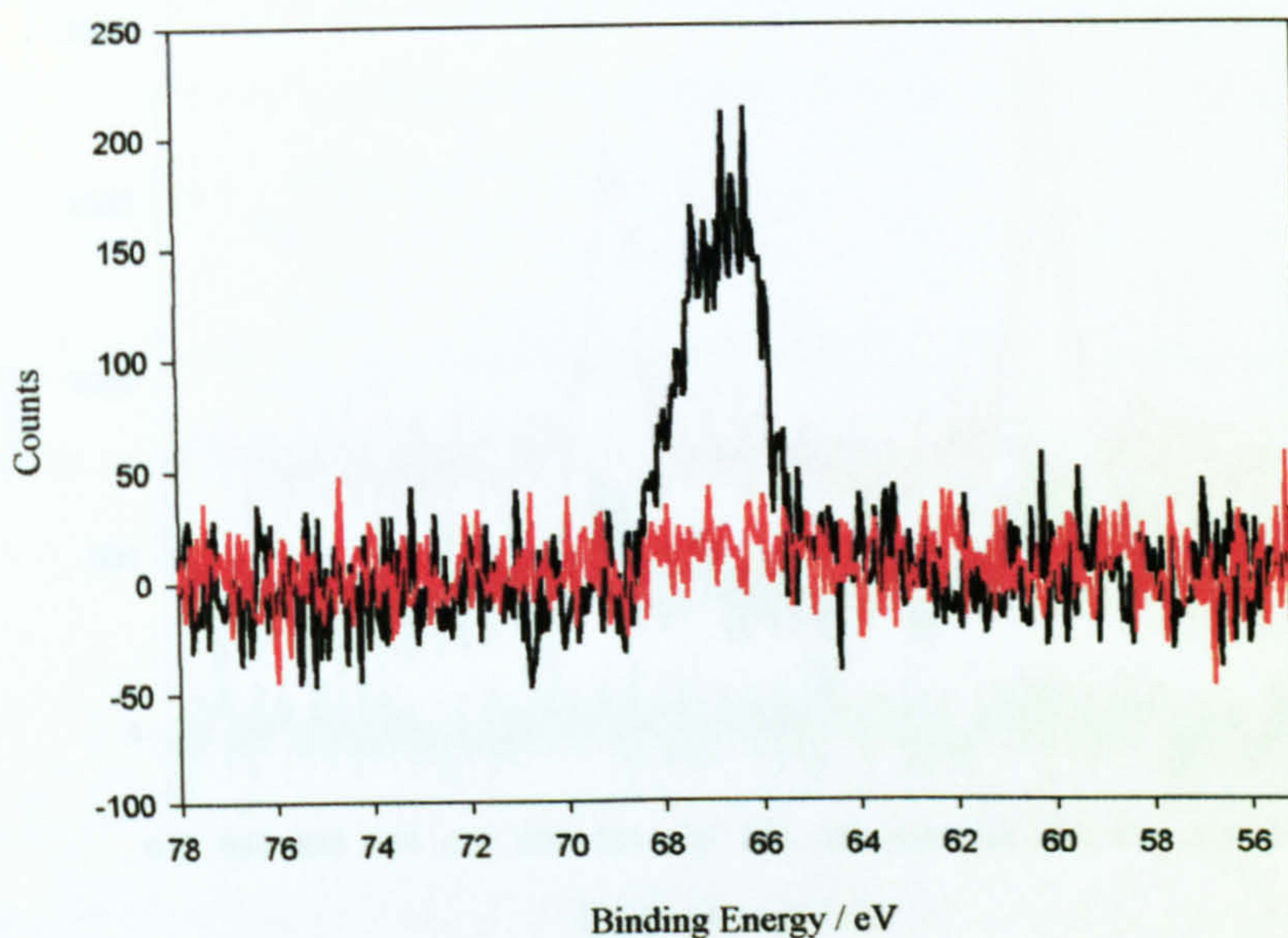




**Figure 8.6:** I (3d) XPS spectra of poly(PFP) (black line), hydrolysed poly(PFP) (red line) and PPG-800 modified poly(PFP) (green line) after reaction for 1 hour with 0.1 mg/ml Iodo-streptavidin and buffer solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of 20 mVs<sup>-1</sup> and with electrode area of 1 cm<sup>2</sup>. PPG-800 modified poly(PFP) was produced by reacting the polymer with 1 mM PPG-800 and DMSO solution.

To investigate whether adsorption can be further reduced poly(PFP) films were modified with medium-chain PPG for the initial studies. For comparison, both the poly(PFP) and the PPG modified films were immersed in a buffer solution containing brominated cytochrome c. From Figure 8.7 a decrease in Br (3d) signal can be observed for the PPG modified poly(PFP) film. This suggests that the attachment of this group onto the polymer backbone directly influence the ability of species to absorb onto the surface. Further data was collected for these PPG modified film after reaction with Iodo-HRP, Iodo-BSA, Iodo-cytochrome c and Iodo-streptavidin solutions and can be seen in Figures 8.4, 8.5 and 8.6.

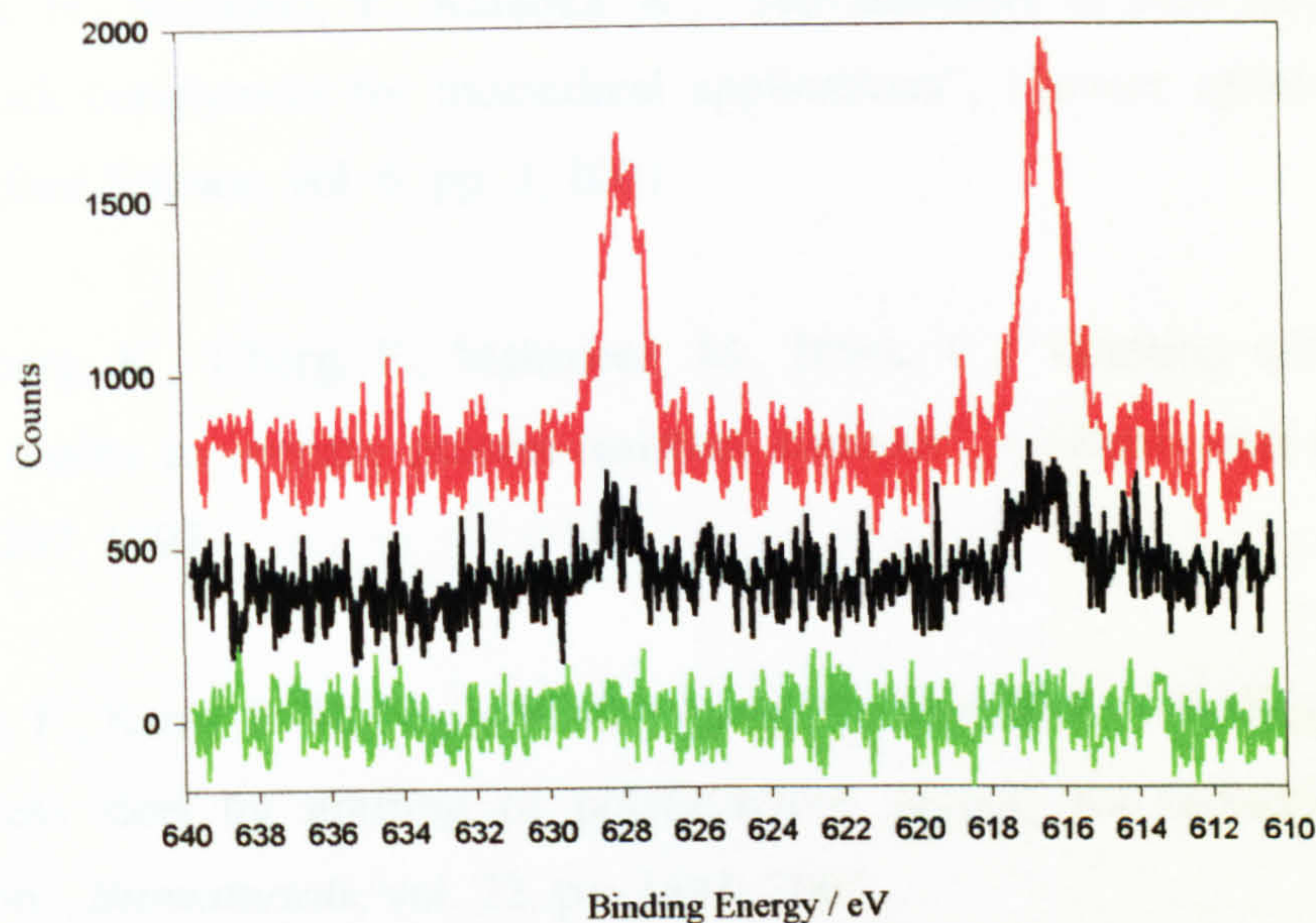




**Figure 8.7:** Br (3d) XPS spectra of poly(PFP) (black line) and PPG-800 modified poly(PFP) (red line) after reaction with  $0.1 \text{ ml}^{-1}$  buffer solution of brominated cytochrome c. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . PPG-800 modified poly(PFP) was produced by reacting the polymer with 1 mM PPG-800 and DMSO solution.

It was reported that the inhibition of protein adsorption was achieved on modified surfaces depending on the PPG molecular weight or chain density<sup>1, 6</sup>, therefore, experiments were carried out to establish whether the prevention of NSB could be optimised by changing the molecular weight of the PPG polymers used. The three examples of PPG modified films were, therefore, immersed into buffer solutions containing the labelled biomolecules. It was established that increasing the PPG chain length would decrease the amount of NSB that would occur. Figure 8.8 shows the I (3d) spectra after the PPG modified polymers were reacted with iodinated species for 1 hour. There is a decrease in iodine signal as the molecular weight of the PPG polymer increases. There was approximately 75 % more iodine signal present in the spectra of the short-PPG polymer than that of the medium-PPG polymer. There is no iodine signal present when the long PPG chain is used.





**Figure 8.8:** I (3d) XPS spectra of PPG-130 (red line), PPG-800 (black line) and PPG-1900 (green line)- modified poly(PFP) films after reaction with  $0.1 \text{ ml}^{-1}$  Iodo-streptavidin and buffer solution. Poly(PFP) was produced from solution containing 20 mM PFP, 0.1 M TEAP and MeCN at a scan rate of  $20 \text{ mVs}^{-1}$  and with electrode area of  $1 \text{ cm}^2$ . Each PPG modified poly(PFP) was produced by reacting the polymer with the appropriate 1 mM PPG and DMSO solution.

### 8.3 Summary

It was possible to modify poly(PFP) with amine terminated PPG species and consequently amide bond detection was possible using RAIRS. The results of adsorption experiments on polymer films modified with different chain lengths of PPG molecules have shown that the PPG of molecular weight of 1900 are required to reduce non specific binding to negligible level.

It was noted that by hydrolysing the poly(PFP) film, non specific binding reduces significantly. Changing the chemical structure of the polymer backbone can change the degree of interaction with biomolecules.



## 8.4 References

1. Otsuka, H., Nagasaki, Y., Kataoka, K., "Self-assembly of poly(ethylene glycol)-based block copolymers for biomedical applications", *Current opinion in Colloid and Interface Science*, vol. 6, pp. 3, 2001.
2. Holmberg, K., Tiberg, F., Malmsten, M., Brink, C., "Grafting with hydrophilic polymer chains to prepare protein-resistant surfaces", *Colloids and Surfaces*, vol. 123, pp. 297, 1997.
3. Zhang, F., Kang, E. T., Neoh, K. G., Wang, P., Tan, K. L., "Surface modification of stainless steel by grafting of poly(ethylene glycol) for reduction in protein adsorption", *Biomaterials*, vol. 22, pp. 1541, 2001.
4. Piehler, J., Brecht, A., Valiokas, R., Lieberg, B., Gauglitz, G., "A high-density poly(ethylene glycol) polymer brush for immobilisation on glass-type surfaces", *Biosensors and Bioelectronics*, vol. 15, pp. 473, 2000.
5. Kingshott, P., Griesser, H. J., "Surfaces that resist bioadhesion", *Current opinion in solid state and materials science*, vol. 4, pp. 403, 1999.
6. Malmsten, M., Emoto, K., Van Alstine, J. M., "Effect of Chain density on Inhibition of Protein Adsorption by Poly(ethylene glycol) Based coatings", *Journal of Colloid and Interface Science*, vol. 202, pp. 507, 1998.
7. Schneider, B. H., Dickinson, E. L., Vach, M. D., Hoijer, J. V., Howard, L. V., "Highly sensitive optical chip immunoassays in human serum", *Biosensors and Bioelectronics*, vol. 15, pp. 13, 2000.
8. Ista, L. K., Fan, H., Baca, O., Lopez, G. P., "Attachment of bacteria to model solid surfaces: oligo(ethylene glycol) surfaces inhibit bacterial attachment", *FEMS Microbiology Letters*, vol. 142, pp. 59, 1996.



9. Chen, Y., Kang, E. T., Neoh, K. G., Wang, P., Tan, K. L., "Surface modification of polyaniline film by grafting of poly(ethylene glycol) for reduction in protein adsorption and platelet adhesion", *Synthetic Metals*, vol. 110, pp. 47, 2000.



# Chapter 9

## *Conclusion and Further Work*

### *9.1 Conclusion*

The main objective of the research described in this thesis is to develop a generic system, which enables the systematic modification of conducting polymers, such that they can be used in analytical biotechnology. The development of a multi analytical device would be of advantage, which encouraged research into a method to control the reaction of the conducting polymer with reactive species.

Initial results involve determining characteristics of the polymer to be used for the research. Electrochemical techniques were used to prepare poly(PFP) homopolymers and poly(Py/PFP) copolymers.

Both XPS and RAIRS were used to analyse the polymer films before and after modifications were performed. XPS gives information on the interface of each reacted polymer, indicating any changes in polymer composition. RAIRS was mainly used to ensure the desired aminolysis reaction has occurred and also to establish if there was any unwanted side reactions (e.g. hydrolysis).

In addition, it has been illustrated that spectral decomposition of C (1s) XPS signals from electrodeposited polymer interfaces can be used to determine the composition of various Py/PFP copolymer films.

The initial reactions performed involve the homopolymer and copolymer films with ferrocene ethylamine and hexylamine. For the immobilisation reaction to occur the



amine terminus of the reactive species reacts with the activated ester contained within the polymers. RAIRS was subsequently used to detect the resulting amide bonds, as amide bands at 1540 and 1680  $\text{cm}^{-1}$  are apparent in the spectra. By using Py/PFP copolymer films the amount of ferrocene immobilised within the film can be controlled. This was obvious from both XPS and electrochemical results.

The reaction kinetics was also described and as ferrocene ethylamine has an XPS probe it was used for the experiments performed. It was important that the same growth parameters were used when preparing these polymers as any major variances can lead to a change in reaction rates, polymer microstructure and film thickness, therefore, it was important that the same growth conditions to produce polymer-modified electrodes were used.

As ferrocene ethylamine was not suitable for EQCM experiments, hexylamine was used to monitor the immobilisation reaction using EQCM. These experiments show that when hexylamine reacts with poly(PFP) the frequency measurements increase as the leaving PFP group has a higher molecular weight than hexylamine. It was noted that there was a change in reaction rates for samples of poly(PFP) film grown using different electrochemical growth parameters.

In addition the reaction of poly(PFP) with ferrocene ethylamine and hexylamine could be controlled by oxidatively doping the polymer. Moreover, the substitution reaction can still occur on a polymer that has previously been doped i.e. the inhibition is not permanent. This method of reactivity control could be useful for the patterning of various species on microfabricated arrays and reports the initial investigations of preparation of the polymer that could possibly be used as a matrix for the production of a multianalytical biosensor.

Results to date do not establish a mechanism for the inhibition of these reactions, however mechanistic possibilities may include: changes in bond strength of the activated ester; conformation changes of the conducting polymer backbone.



The same control could be used for the reaction of cystamine and poly(PFP) and, furthermore, it was possible to use the cystamine modified polymer as a template for further modification. Initially, XPS measurements was used to determine an appropriate solvent, which allows an organic coupling reaction between poly(PFP) and solution species (cystamine) to proceed. The cystamine-modified polymer was then used as a template for metal ion chelation. A combination of XPS and QCM measurements has enabled to show that the binding of a “soft” metal species, Pt, from aqueous solution to thiol groups within the polymer is possible. The resulting complex retains chloride ligands, which allow further nucleophilic substitution with a variety of species. This was investigated using species, which have an easily detectable XPS probe, and results show substitution of the chloride with amine and thiol terminated species is possible.

The cystamine-modified film was also reacted with I-PEO-acetylbiotin and then with streptavidin-enzyme conjugate. This was possible as the nucleophilic substitution of the iodo group with thiol group, present in the polymer, occurred. This technique also provides the opportunity to selectively pattern enzymes on electrodes however initial experiments were unsuccessful as non specific binding occurred on the electrodes. The method of labelling the proteins with unique XPS labels, which have a high sensitivity, has shown to be of value in evaluating whether successful immobilisation on the polymer interface has occurred.

Labelled proteins were also used in experiments that involved the reaction of activated ester present within poly(PFP) with amine terminated biotin species and then, subsequent reaction with streptavidin-enzyme conjugates. The binding of the streptavidin conjugates on the biotinylated polymers was then probed using XPS. These studies have indicated that when using conjugated enzymes the entire bound enzyme is present through the streptavidin interaction with the substrate. When non-conjugated enzymes were used there was visible XPS signal detected thus suggesting that non specific adsorption does not occur.



Furthermore, whilst investigating a technique to reduce non specific binding poly(PFP) was modified with amine terminated PPG species. The results of adsorption experiments on polymer films modified with different chain lengths of PPG molecules have shown that the PPG of molecular weight of 1900 are required to reduce non specific binding to negligible level.

It was noted that by hydrolysing the poly(PFP) film, non specific binding reduces significantly. Changing the chemical structure of the polymer backbone can change the degree of interaction with biomolecules.

## 9.2 *Further Work*

The basis of this research was to develop a polymer system that could be used in the development of a biosensor. Some of the techniques used to modify the poly(PFP) film have been developed further by members of the Bioelectronics Department in Glasgow. For example, poly(PFP) was deposited on an interdigitated electrode array and subsequently modified with biotin and then streptavidin-HRP conjugate. Consequently it was possible to detect hydrogen peroxide using ferrocene as a redox mediator for the HRP catalysed reaction. To date research is being performed to develop some of the modification techniques used in this research to produce a multi analytical device.

